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**OXIDATION AND BLEACHING SYSTEM WITH ENZYMATICALLY
PRODUCED OXIDIZING AGENTS**

It is known from a number of literature references and review articles, for example from "Preparative Biotransformations" by S.M. Roberts, K. Wiggins and G. Casy, J. Wiley & Sons Ltd, that enzymes such as certain lipases are capable of forming epoxides via the formation of peroxy acids (perfatty acids). For example, in the lipase system (from *Candida antarctica*), with continuous addition of H_2O_2 and in the presence of certain fatty acids, for example tetradecanoic (myristic) acid or dodecanoic (lauric) acid, from cyclooctene the corresponding epoxide is produced. It is also known that manganese peroxidases + unsaturated fatty acids can generate peracids which in turn can act as a H_2O_2 source for manganese peroxidases (literature: B.W. Bogan et al., Applied and Environmental Microbiology, vol. 62, No. 5, pp. 1788-1792).

There are also a few patents that describe the formation of peracids with the aid of haloperoxidases.

It is also known that dimethyldioxirane can be generated in situ from peracids or salts of peracids (such as Oxone) and acetone as the simplest ketone. Ketones other than acetone are also used. Dioxiranes can also be prepared as pure substances before they are used as oxidants, but their stability is problematical (WO 92/13993).

Moreover, it is known from Canadian Patent 1 129 162, US 5, 034 096 and WO 96/13634 that certain metal ions, for example $Mo^{6+} + H_2O_2$ and nitrilamides + H_2O_2 and dicyandiamides + H_2O_2 are capable of generating dioxiranes chemically from H_2O_2 . In this case, surprisingly, certain combinations are possible with the enzyme components system (ECS) of the present invention (see below), namely the enzymatic generation of activated oxygen species e.g. dioxiranes can be further enhanced.

These very strong and highly selective oxidants can be used in many oxidation reactions (for example epoxide reactions etc). Recently, it has been proposed to use them, in particular, as bleaching agents in the cellulose /pulp industry. Because of the dangerous preparation and high cost, this proposal has not found acceptance (WO 92/13993).

The object of the present invention is to provide a highly selective oxidation or bleaching system for use in cellulose /pulp bleaching or high yield wood pulp bleaching, in the oxidative treatment of wastewater of all kinds, in the preparation of wood-based composites, as an enzymatic deinking system, as an oxidant in organic synthesis, in coal liquefaction, as bleaching system in detergents and as a bleaching agent or oxidant in the textile industry (for example in stone washing and fabric bleaching). Said system does not present many of the drawbacks of purely chemical systems (for example environmental pollution problems) or of enzymatic systems (which often show inadequate performance and are very costly).

Surprisingly, we have now found, that an oxidation system which contains certain lipases, oxidants such as H_2O_2 , certain fatty acids and certain ketones resulted, for example, in bleaching of cellulose/pulp while at the same time the kappa number

(delignification) was markedly reduced, i.e. it could be clearly demonstrated, surprisingly, that when the appropriate optimal components were present in an optimum proportion and concentration relative to each other, it was possible to achieve in the aforesaid cellulose/pulp bleaching a bleaching action comparable to that of dioxirane-forming chemical systems.

Surprisingly, it was also possible to demonstrate considerable bleaching action in the bleaching of high yield wood pulp, in the bleaching of pulps after a deinking processes, in oxidative polymerization of lignin and/or lignin-like substances and in the oxidative treatment of wastewater of all kinds, such as wastewater from high yield wood pulp preparation (groundwood, refiner pulp), from the cellulose/pulp industry and dye-contaminated wastewater, for example from the textile industry. For most of these wastewaters, besides the decolorization and oxidation and thus the "destruction" of environmental pollutants, the polymerization of lignins is the preferred application, because it causes a marked increase in molecular size and permits an easier and substantially less expensive precipitation of these polymers and thus their elimination from COD considerations.

Surprisingly, we were also able to demonstrate this oxidative polymerization of lignin and/or lignin-like substances in the preparation of wood-based composites (binder and/or adhesive preparation) by oxidative polymerization of the polyphenylpropanes present. Moreover, surprisingly, we were able to demonstrate removal of printing inks in the deinking process (probably occurring by swelling of the lignin-containing waste paper fibers). Surprisingly, we were also able to observe coal liquefaction properties in the treatment of lignite or anthracite. Moreover, also surprisingly, we found a marked and selective oxidation power in the use as "oxidant" in organic synthesis, high bleaching power when used as bleaching additive to detergents, in the general bleaching of textile fabrics and as special bleach when used in the stone washing processes, namely as a replacement for mechanical color removal and/or as postbleach in these processes. It seems possible, that the responsible oxidants(s), for example, generated from the present ketones and peracids are dioxiranes which in the above-indicated applications serve as oxidants or bleaching agents either alone or in combination with the peracids formed.

The foregoing objective is reached also by providing an enzyme component system (ECS) according to the invention which contains one or more lipases, preferably from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases, preferably from the group of amidases (3.5.1.4) (**system component 1**) which from one or more fatty acids present (preferably C₆- C₂₆ and particularly C₈ - C₁₆ fatty acids) (**system component 2**) and in the presence of an oxidant such as a peroxide, preferably H₂O₂ (**system component 3**) can produce peracids and in the presence of ketones as additional component (**system component 4**), for example, dioxiranes.

Description of Various Applications of the Enzyme Component System (ECS) of the Invention

I) Use in the bleaching of cellulose/ wood pulp.

Use:

- a) in the treatment of, primarily, wood pulp wastewater in the pulp and paper industries, and**
- b) of wastewater in other industries.**

III) Use in the preparation of lignin solutions or gels, of the corresponding binders/adhesives and of wood-based composites.

IV) Use as enzymatic deinking system.

V) Use as oxidation systems in organic synthesis.

VI) Use in coal liquefaction.

VII) Use as bleaching agent in detergents.

VIII) Use in the bleaching/decolorization of textile fabrics.

D) Use of the Enzyme Component System (ECS) of the Invention in the Bleaching of Cellulose/Wood Pulp

Wood pulp is currently produced mainly by the sulfate and sulfite processes. By both processes, pulp is made by cooking at high temperature and under pressure. The sulfate process involves the addition of NaOH and Na₂S, whereas the sulfite process uses Ca(HSO₃)₂, + SO₂, although the sodium and ammonium hydrogen sulfite salts are currently used because of their higher solubility.

The main objective of all processes is the removal of lignin from the plant material, wood or annual plants used.

The lignin, which together with the cellulose and hemicellulose forms the main constituent of the plant material (stalks and stems), must be removed, because it is otherwise not possible to produce nonyellowing, mechanically highly resistant papers.

The processes for making high yield wood pulp involve the use of stone grinders (groundwood) or of refiners (TMP = thermomechanical pulp) which after an appropriate pretreatment (chemical, thermal or thermechemical) defibrillate the wood by milling.

These wood pulps still contain most of the lignin. They are used primarily for the production of newspapers, magazines etc.

The possibilities of using enzymes for lignin degradation have been under investigation for several years. The mechanism of action of such lignolytic systems was discovered

only a few years ago, when it became possible to obtain sufficient amounts of enzymes from the white rotting fungus *Phanerochaete chrysosporium* by use of proper culturing conditions and the addition of inductors. This is how the hitherto unknown lignin peroxidases and manganese peroxidases were detected. Because *Phanerochaete chrysosporium* is a very effective lignin degrader, attempts have been made to isolate its enzymes and use them in purified form for lignin degradation. This was unsuccessful, however, because it was found that the enzymes primarily cause repolymerization of lignin and not its degradation.

The same is true for other lignolytic enzyme species, such as the laccases which degrade lignin oxidatively with the aid of oxygen rather than hydrogen peroxide. It was found that similar processes are at work in all cases, namely that radicals are formed which then react with each other causing the mentioned polymerization.

Currently, there are only processes based on the use of in-vivo systems (fungal systems). Optimization attempts were directed mainly toward **biopulping and biobleaching**.

By biopulping is meant the treatment of wood chips with live fungal systems. There are two kinds of application forms:

1. Pretreating the wood chips before charging them to the refiners or milling for the purpose of saving energy in high yield wood pulp production (for example, TMP or groundwood). Another advantage is the usually achieved improvement of mechanical properties of the stock, and a drawback is that the final brightness is worse.
2. Pretreating the wood chips (softwood/hardwood) before wood pulp cooking (kraft process, sulfite process). Here, the objective is to reduce the amount of digestion chemicals, to improve digestion capacity and extended cooking. The advantages include improved kappa number reduction following digestion compared to digestion without pretreatment.

The drawbacks of these processes are clearly their long treatment times (several weeks) and particularly the unsolved problem of risk of contamination during the treatment, if it is desired to omit the uneconomical sterilization of the wood chips.

Biobleaching also uses in-vivo systems. Before bleaching, the digested pulp (softwood/hardwood) is inoculated with the fungus and treated for a period of days or weeks. Only after such a long treatment time is it possible to observe a drop in kappa number and a significant improvement in brightness, so that the process is uneconomical for implementation in current bleaching sequences.

Another application, mostly carried out with immobilized fungal systems, is the treatment of pulp production wastewaters, particularly bleaching plant wastewaters, for the purpose of decolorizing them and reducing the AOX value (reducing the amount of chlorinated compounds in the wastewater, compounds which were used for chlorine or chlorine dioxide bleaching). It is also known to use hemicellulases and particularly xylanases and mannases as bleach boosters.

These enzymes act mainly on the reprecipitated xylan, which after the cooking process partly covers the residual lignin, for the purpose of degrading it and thus improving accessibility to the lignin of the bleaching chemicals (primarily chlorine dioxide) used in the subsequent bleaching sequences. The savings in bleaching chemicals demonstrated in the laboratory have been confirmed on a large scale only to a limited extent so that this type of enzyme must also be classified as a bleaching additive.

Patent application PCT/EP 87/00635 describes a system for removing lignin from lignin-cellulose- containing material with simultaneous bleaching. The system is based on the use of lignolytic enzymes from white rotting fungi with the addition of reducing agents, oxidants and phenolic compounds as mediators.

According to DE 4 008 893 C2, in addition to the redox system, mimicking substances are added which simulate the active center (prosthetic group) of lignolytic enzymes. In this manner, a marked improvement in performance is achieved.

According to patent application PCT/EP 92/01086, additional improvement is achieved by use of a redox cascade with the aid of phenolic or nonphenolic aromatics "balanced" in terms of their oxidation potential.

All three processes are limited in regard to their applicability on an industrial scale in that they must be used at low wood pulp consistency (up to a maximum of 4%) and, in the case of the last two applications also by the risk of leaching out metals when chelating agents are used, the metals possibly causing peroxide decomposition in the subsequent peroxide bleaching stages.

WO 94/12619, WO 94/12620 and WO 94/12621 disclose processes in which the peroxidase activity is increased with enhancers. Enhancers are characterized in WO 94/12619 in terms of their half-life.

According to WO 94/12620, enhancers are characterized by the formula $A=N-N=B$ where N means nitrogen, A and B are defined cyclic groups. According to WO 94/12620, enhancers are organic chemicals containing at least two aromatic rings of which at least one is substituted with defined groups.

All three patent applications concern dye transfer inhibition and the use of enhancers together with peroxidases as detergent additives or detergent compositions used in the detergent sector.

Although the applicability to lignin is mentioned in the specification of the applications, our own tests with the substances actually disclosed in these applications have shown that the claimed mediators are ineffective in increasing the bleaching action of peroxidases in the treatment of lignin-containing materials!.

WO 94/29510 and WO 96/18770 describe a process for enzymatic delignification whereby the enzymes are used together with mediators. The mediators disclosed are, in general, compounds characterized by the structure NO-, NOH- or NRNOH.

Among the mediators disclosed in WO 94/29510 and WO 96/18770, 1-hydroxy-1H-benzotriazole (HOBt) gave the best delignification results. HOBt, however, has several drawbacks:

- it is available only at a relative high price and in insufficient quantities,
- under delignification conditions, it reacts forming 1 H-benzotriazole and other colored products, this compound shows relatively low degradability and could, in large amounts, present a pollution problem,
- to a certain degree, it harms the enzymes,
- its delignification velocity is not very high.

Other mediator of the described NO-, NOH- and HRN-OH type do not show most of these drawbacks, but still have the disadvantage that a relatively large amount of chemicals must be used and, particularly, that because of their physiological reactivity they may not be entirely harmless (mostly because of NO- radical formation).

It is therefore desirable to provide systems for modifying, degrading or bleaching lignin, lignin- containing materials or similar substances, which do not have the said drawbacks or present them only to a minor degree.

Quite surprisingly, we have now found that when the enzyme component system (ECS) of the invention is used, similar or better delignification and bleaching results are achieved compared to the abovesaid oxidoreductase-mediator systems, and the said drawbacks are negligible.

In other words, according to the invention, the foregoing objective is reached by providing an enzyme component system (**ECS**) according to which contains one or more lipases, preferably from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases, preferably from the group of amidases (3.5.1.4) (**system component 1**) which from one or more fatty acids present (preferably C₆- C₂₆ and particularly C₈ - C₁₆ fatty acids) (**system component 2**) and in the presence of an oxidant such as a peroxide, preferably H₂O₂ (**system component 3**) can produce peracids and in the presence of ketones as additional component (**system component 4**), for example, dioxiranes.

Unlike most enzymes, oxidases and peroxidases exhibit low substrate specificity, namely they can convert a wide range of substances, usually of phenolic nature. Without mediators, oxidases as well as many peroxidases show the tendency to polymerize phenolic substances via free radical-induced polymerization, a property which is attributed, for example, to laccase, belonging to the group of oxidases, also in nature. The ability to polymerize appropriate substances, for example lignins, namely to increase the size of the molecules involved by "coupling reactions", can be utilized, for example, for the treatment of lignin- containing wastewaters in the paper industry such as TMP wastewater (wastewater from the production of thermomechanical pulp by means of refiners) and of grinder wastewater from mechanical wood pulping units.

The water-soluble lignin compounds (polyphenolpropanes) contained in these wastewaters are mainly responsible for the high COD (chemical oxygen demand) and cannot be removed by conventional technology. In the water treatment plant and in the downstream waters, they are not degraded at all or they are degraded only very slowly. At very high concentrations, these compounds can even inhibit the bacteria in a water treatment plant and thus create problems.

In this application, the enzyme action can be observed immediately by a rapid development of turbidity in the wastewater being treated, caused by an enlargement and thus insolubilization of lignin molecules. The target molecules (polymerized lignin) thus enlarged in molecular weight by enzymatic catalysis can be removed by appropriate treatments (by flocculation, by precipitation with, for example, aluminum sulfate/sodium aluminate, optionally in the presence of cationic or anionic polyelectrolytes or by sedimentation). The wastewater then shows a markedly reduced COD. Upon disposal, such wastewater causes less pollution, namely it increases the certainty of remaining below the permissible COD limits. Thus is particularly important for not infrequently used "procedures" run at the limit.

For this treatment, for example with laccase, the cost of removing the reaction products of the enzymatic treatment by flocculation, sedimentation or precipitation or a combination of several such methods constitutes by far the predominant part of the overall cost of the process.

We have now found, quite surprisingly, that when the enzyme component system (ECS) of the invention is used by employing a special combination of the components, much higher efficiency than be attained than with the above-described enzymatic systems. This means that the process according to the invention represents a substantially improved system compared to the aforesaid systems employing oxidoreductases (such

alizarin, 5-amino-2-hydroxybenzoic acid, 3-aminophenol, pyrocatechol, 2,2-bis(4-hydroxyphenyl)- propane, bis(4-hydroxyphenyl)methane, quinalizarin, 4-chloro-1-naphthol, coniferyl alcohol, 2,4-di- aminophenol dihydrochloride, 3,5-dichloro-4-hydroxyaniline, 1,4-dihydroxyanthraquinone, 2,2-di- hydroxybiphenyl, 4,4-dihydroxybiphenyl, 2,3-dihydroxynaphthalene, 2,6-diisopropylphenol, 3,5-di- methoxy-4-hydroxybenzhydrazine, 2,5-ditert.butylhydroquinone, 2,6-ditert.butyl-4-

methylphenol, 4-hydroxybiphenyl, 2-hydroxydiphenylmethane, 2-(2-hydroxyphenyl)benzothiazole, 5-indanol, 2-iso-propoxyphenol, 4-isopropyl-3-methylphenol, 5-isopropyl-2-methylphenol, 4-isopropylphenol, lauryl gallate, 2-naphthol, 4-nonylphenol, 3-(pentadecyl)phenol, 2-propylphenol, 4-propylphenol, purpurine, pyrogallol, 4-(1,1,3,3-tetramethylbutyl)phenol, 1,2,4-trihydroxybenzene, 2,4,6-trimethylphenol, 2,3,5-trimethylphenol, 2,3,6-trimethylphenol, 3,4,5-trimethylphenol, 6,7-dihydroxy-4-methyl coumarin, 2-(2-hydroxyethoxy)benzaldehyde, 1-naphthol, nordihydroguaiaretic acid, octyl gallate, silibinin, 3,4,6-trihydroxybenzoate-octylester, 2,4,6-tritert.butylphenol, 2,4-ditert.butylphenol, 2,6-dichlorophenol, indophenol, ethoxyquin, 1-aminoanthraquinone, 2-amino-5-chlorobenzophenone, 4-aminodi-phenylamine, 7-amino-4-hydroxy-2-naphthalenesulfonic acid, 2-(4-aminophenyl)-6-methylbenzothiazole, benzanthrone, trioctyl trimellitate, trans-chalcone, bis(4-aminophenyl)amine sulfate, 2,2'-ethylidenebis(4,6-ditert.butylphenol), 2,2-bis(2,6-dibromo-4-(2-hydroxyethoxyphenyl)propane, bis(3,5-ditert.butyl-4-hydroxyphenyl)methane, 2,2-bis(3,5-dichloro-4-hydroxyphenyl)propane, Bismarck Brown Y, 1-bromophthalein, 4-butylaniline, 2-tert.butyl-5-methylphenol, 1-chloroanthraquinone, 2-chloroanthraquinone, triallyl 1,3,5-benzenetricarboxylate, 1,1,1-tris(hydroxymethyl)propane, tri-methacrylate, pentaerythrityl triacrylate, 1,2,4-trivinylcyclohexane, trans,cis-cyclododeca-1 5,9-tri-ene, pentaerythritol tetrabenzoate, 4,4'-methylenebis(2,6-ditert.butylphenol), 4,4'-isopropylidene-bis(2,6-dichlorophenol), 4,4'-isopropylidene-bis(2,6-dibromophenol), 4,4'-isopropylidene-bis[2-(2,6-dibromophenoxy)ethanol, 2,2'-ethylidene-bis(4,6-ditert.butylphenol), 3-tert.butyl-4-hydroxy-5-methylphenol, 5-tert.butyl-4-hydroxy-2-methylphenol, syringaldazine, 4,4'-dimethoxytriphenylmethane and di-sec.butylphenol.

Also particularly preferred are compounds with several hydroxyl groups, such as:

ellagic acid, gallic acid, gallein, gallangin, myoinositol, morin, nitranilic acid, phenolphthalein, purpurin, purpurogallin, quinizarin, chrysazin, quercitin, quinydrone, chloranilic acid, carmine, rhodizone acid, croconic acid, meilitic acid, hematoxylin, 9-phenyl-2,3,7-trihydroxy-6-fluorene, 9-methyl-2,3,7-trihydroxy-6-fluorene, tetrahydroxy-p-benzoquinone, 2,2',4,4'-tetrahydroxybenzophenone, Pyragallol Red, 1-nitrophloroglucinol, 1,4-dihydroxyanthraquinone, 5,8-dihydroxy-1,4-naphthoquinone, hexa-oxocyclohexane octahydrate, 5,7-dihydroxyflavanone, 3',4'-dihydroxyflavanone, glyoxal hydrate, 1,3,5-tris(2-hydroxyethyl)isocyanuric acid, quinalizarin and 2,4,5-trihydroxybenzamine.

III) Use of the Enzyme Component System of the Invention in the Preparation of Lignin Solutions or Gels, of the Corresponding Binders/Adhesives and of Wood-Based Composites

The object of the present invention is to provide a process for enzymatic polymerization and/or modification of lignin or lignin-containing materials, for example for use in the production of wood compositions or wood-based composites such as, for example, fiber board from disintegrated wood or particle board from wood chips or wood pieces (chipboard, plywood, wood composite beams).

We have now found, quite surprisingly, that here, too, the enzyme component system (ECS) of the invention shows much better performance compared to the prior-art enzymatic systems for the polymerization and/or modification of lignin and/or lignin-containing materials.

To this end, the enzyme component system of the invention is brought together with lignin (for example, with lignosulfates and/or unevaporated or evaporated sulfite waste liquor and/or sulfate lignin --> kraft lignin, for example induline) and/or with lignin-containing material. The lignin and/or the lignin-containing material can either be preincubated at an elevated pH, namely above pH 8 and preferably at a pH between 9.5 and 10.5, at 20 to 100 °C (preferably at 60 to 100 °C) after which the pH is reduced to below pH 7, depending on the optimum pH range for enzyme activity of the component system (ECS) or, if the activity optimum of the enzyme component system (ECS) is on the alkaline side, the ECS and the lignin and/or the lignin-containing material are brought together immediately, without pretreatment. The purpose of the pretreatment or treatment under alkaline pH conditions is to utilize the substantially easier solubilization of lignin at these higher pH values. This is a major advantage for the use according to the invention, because it is thus possible to work without an organic solvent.

The polymerizing and/or modifying action of the enzyme component system can be additionally enhanced by addition of certain chemical polymerization catalysts, for example polydiphenylmethane diisocyanate (PMDI) and other polymerization catalysts

used also for the polymerization of lignin in lignin-containing wastewaters. Such substances consist of phenols, phenol derivatives or other polycyclic phenolic compounds with a number of oxidizable hydroxyl groups, as already indicated hereinabove (wastewater treatment).

IV) Use of the Enzyme Component System (ECS) as an Enzymatic Deinking System

In principle, by deinking, which is currently always run in a conventional manner by flotation, is meant a two-step process.

Its objective is to remove printing ink and other dye particles from the waste paper. The waste paper used in most cases is paper collected domestically and consists mainly of newspapers and magazines.

In the first treatment step, the dye particles adhering to the paper fibers are removed primarily by mechanical/chemical means. This is accomplished by "recycling" the paper as a uniform fibrous slurry, namely by disintegrating (comminuting) the waste paper in pulpers, drums or the like with simultaneous addition of chemicals capable of enhancing removal and preventing yellowing and thus also acting as bleaching chemicals, namely sodium hydroxide solution, fatty acid, water glass and hydrogen peroxide (H_2O_2). Here, the fatty acid acts as a fiber dye particle collector and in the second treatment step, the flotation, also as foaming agent.

After the waste paper has been disintegrated and the said chemicals have been allowed to act for a certain length of time, the flotation is carried out in special flotation vessels by injecting air. During this process, the dye particles adhere to the foam bubbles and are removed together with the bubbles. The dye is thus separated from the paper fibers. Currently, this operation is preferably carried out at a neutral pH, which makes it necessary to use certain detergents in place of the fatty acids.

It is known from the literature (WO 91/14820, WO 92/20857) to use an oxidoreductase or laccase system characterized primarily by the addition of special substances which cause the optimum pH for the action of laccase obtained from *Trametes versicolor*, which normally is in the range of about pH 4-5, to shift into the slightly alkaline range (pH 8 to 8.7). This, on the one hand, is an important prerequisite for use in the deinking system because of the $CaSO_4$ problems arising below pH 7 and, on the other, does not optimize the action of laccase in the polymerizing or depolymerizing sense, but only produces a certain swelling of the fibers. Such swelling (which is one of the main actions of sodium hydroxide in purely chemical deinking systems) is a primary performance characteristic of the dye-removing mechanism.

The only other additives required for this enzymatic system employing oxidoreductases are the detergents needed to produce foam. Nearly all suitable detergents also exert a dye-removing action. Moreover, in conventional deinking systems the use of sodium hydroxide and peroxide improves brightness as a result of the bleaching action of these

chemicals. This bleaching action cannot be achieved with the prior-art enzyme system because of the nature of the system.

We have now found, quite surprisingly, that by appropriate selection of the components the enzyme component system (ECS) of the invention exceeds the efficiency of other enzymatic deinking systems, particularly those with oxidoreductases and those applied to lignin-containing deinked pulp and at least in part compensates for the advantage of bleaching with purely chemical systems. This means that it is possible to provide a system offering environmentally friendly deinking under neutral pH conditions and thus better post-bleaching, better pulp properties etc and good performance similar to that of purely chemical systems.

In other words, according to the invention, the foregoing objective is reached by providing an enzyme component system (**ECS**) according to which contains one or more lipases, preferably from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases, preferably from the group of amidases (3.5.1.4) (**system component 1**) which from one or more fatty acids present (preferably C₆- C₂₆ and particularly C₈ – C₁₆ fatty acids) (**system component 2**) and in the presence of an oxidant such as a peroxide, preferably H₂O₂ (**system component 3**) can produce peracids and in the presence of ketones as additional component (**system component 4**), for example, dioxiranes.

In this case, a further improvement of printing ink removal can be attained by the aforesaid addition of special substances mostly of a phenolic nature and, in particular, containing several hydroxyl groups, which are also used as polymerization catalysts in enzymatic wastewater treatment and general polymerization reactions, as in the production of binders/adhesives from lignin or lignin-containing substances primarily for the preparation of wood-based composites.

V) Use of the Enzyme Component System (ECS) of the Invention as an Oxidation System in Organic Synthesis

Recently, enzymes have increasingly been used for chemical reactions in organic synthesis. A few examples showing a variety of oxidative reactions that can be carried out with enzymatic systems can be found in: Preparative Biotransformations (Whole Cell and Isolated Enzymes in Organic Synthesis), S.M. Roberts, K. Wiggins and G. Casy, J. Wiley & Sons Ltd, 1992/93; Organic Synthesis with Oxidative Systems, H.L. Holland, VCH, 1992; and Biotransformations in Organic Chemistry, K. Faber, Springer Verlag [publisher], 1992:

1) Hydroxylation reactions

- a) Synthesis of alcohols
- b) Hydroxylation of steroids
- c) Hydroxylation of terpenes
- d) Hydroxylation of benzenes
- e) Hydroxylation of alkanes
- f) Hydroxylation of aromatic compounds
- g) Hydroxylation of double bonds
- h) Hydroxylation of nonactivated methyl groups

i) Dihydroxylation of aromatic compounds

2) Oxidation of unsaturated aliphatics

- a) Preparation of epoxides
- b) Preparation of compounds by epoxidation
- c) Preparation of arene oxides
- d) Preparation of phenols
- e) Preparation of cis-dihydrodiols

3) Baeyer-Villiger oxidations

- a) Baeyer-Villiger conversion of steroids

4) Oxidation of heterocycles

- a) Transformation of organic sulfides
- b) Oxidation of sulfur compounds
- c) Oxidation of nitrogen compounds (formation of N-oxides etc.)
- d) Oxidation of other heteroatoms

5) Carbon-carbon dehydrogenation

- a) Dehydrogenation of steroids

6) Other oxidation reactions

- a) Oxidation of alcohols and aldehydes
- b) Oxidation of aromatic methyl groups to aldehydes
- c) Oxidative coupling of phenols
- c) Oxidative degradation of alkyl chains (β -oxidation etc.)
- e) Formation of peroxides or percompounds
- f) Initiation of free-radical induced chain reactions.

Here, too, we found, quite surprisingly, that with the aid of the enzyme component system (ECS) of the invention it is possible to carry out many oxidation reactions exemplified hereinabove, namely that the aforesaid objective can be reached by providing an enzyme component system (ECS) according to the invention which contains one or more lipases, preferably from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases, preferably from the group of amidases (3.5.1.4) (**system component 1**) which from one or more fatty acids present (preferably C_6 - C_{26} and particularly C_8 - C_{16} fatty acids) (**system component 2**) and in the presence of an oxidant such as a peroxide, preferably H_2O_2 (**system component 3**) can produce

VI) Use of the Enzyme Component System (ECS) in Enzymatic Coal Liquefaction

Preliminary studies show that, in principle, lignite and anthracite can be attacked and liquefied by in-vivo treatment with, for example, a white rotting fungus such as *Phanerochaete chrysosporium* (incubation time: several weeks/Bioengineering 8, 4, 1992). The possible structure of anthracite is a tridimensional network of polycyclic, aromatic ring systems with a "certain" similarity to lignin structures. Assumed cofactors besides the lignolytic enzymes are chelating agents (siderophors, such as ammonium oxalate) and biosurfactants.

- 1) Until now, effective coal liquefaction systems are known only as in-vivo systems (with lignin- degrading organisms, particularly white rotting fungi), or as systems employing oxidoreductases plus mediators (laccase-mediator system -> WO 94/29510; WO 96/18770).
- 2) It has been proven that, in principle, white rotting fungi that are capable of degrading lignin in vivo can also liquefy coal in culture.
- 3) Coal: Both lignite and anthracite were formed from wood by chemical/physical "actions"; hence, their chemical structures are at least similar to those occurring in lignin.
- 4) In coal liquefaction with white rotting fungi, we see, on the one hand, an alkalization of the pH during fungal growth "on coal" and, on the other, a secretion of siderophor-like chelators, namely substances known to have a positive effect on coal liquefaction.

The main reason for economical, meaningful industrial coal liquefaction is the industrial demand for alternative liquid sources of energy, especially considering that in the future the quantities of other sources of fossil energy such as oil and gas will be decreasing while at the same time the demand for energy will be increasing, and that other alternatives such as nuclear fusion, among others, will not yet be available.

Here, too, we found, quite surprisingly, that with the aid of the enzyme component system (ECS) of the invention liquefaction of, for example, lignite is possible with better performance than with the conventional enzymatic oxidoreductase systems, namely that the aforesaid objective can be reached by providing an enzyme component system (ECS) according to the invention which contains one or more lipases, preferably from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases, preferably from the group of amidases (3.5.1.4) (**system component 1**) which from one or more fatty acids present (preferably C₆- C₂₆ and particularly C₈ – C₁₆ fatty acids) (**system component 2**) and in the presence of an oxidant such as a peroxide, preferably H₂O₂ (**system component 3**) can produce peracids and in the presence of ketones as additional component (**system component 4**), for example, dioxiranes.

VII) Use of the Enzyme Component System (ECS) as Bleaching Agent in Detergents

Conventional bleaching systems in domestic detergents are unsatisfactory, particularly in the low- temperature range. Below a washing temperature of 60 °C, the standard bleaching agent, i.e., H_2O_2 / sodium perborate/sodium percarbonate must be activated by the addition of chemical bleach activators, such as TAED and/or SNOBS*. Also, the need exists for more highly biodegradable, bio-compatible bleaching systems and systems for low-temperature washing that can be used in small amounts. Whereas enzymes are already being used industrially for protein-starch-fat solution and for fiber treatment in the washing process, no enzymatic system is currently available for detergent bleaching. WO 1/05839 describes the use of different oxidative enzymes (oxidases and peroxidases) to prevent dye transfer. As is known, peroxidases are capable of "decolorizing" different pigments (3-hydroxyflavone and betaine are decolorized by horseradish peroxidase and carotene by peroxidase). Said patent application describes the decolorization (also referred to as bleaching) of textile dyes removed from the laundered goods and present in the washing liquor (conversion of a colored substrate into a noncolored, oxidized substance). In this case, the enzyme is said to have the advantage over, for example, hypochlorite which attacks dyes in or on the fabric, in that the enzyme decolorizes only the dissolved dyes. Hydrogen peroxide or an appropriate precursor generating hydrogen peroxide in situ participates in the catalysis of the decolorization. The enzyme reaction can be enhanced somewhat by addition of other oxidizable enzyme substrates, for example metal ions such as Mn^{++} , halide ions, such as Cl^- or Br^- or organic phenols, such as p-hydroxycinnamic acid and 3,4-dichlorophenol. In this case, it is postulated that short-lived radicals or other oxidized states of the added substrate are formed and are responsible for the bleaching or other modification of the colored substance.

US 4, 077 6768 describes the use of iron porphin, hemin chloride, iron phthalocyanines or derivatives thereof together with hydrogen peroxide for preventing dye transfer. These substances, however, are rapidly destroyed by excess peroxide, and for this reason hydrogen peroxide formation must occur in a controlled fashion.

Processes are known from WO 94/12619, WO 94/12620 and WO 94/12621 whereby the activity of the peroxidase is enhanced by means of enhancers. Such enhancers are characterized in WO 94/12620 in terms of their half-life. According to WO 94/12621, enhancers are characterized by the formula $A = N - N = B$ where N means nitrogen and A and B are defined cyclic groups. According to WO 94/12620, enhancers

* TAED = tetraacetythylenediamine; SNOBS = sodium nonyloxybenzenesulfonate

are organic chemicals containing at least two aromatic rings of which at least one is substituted with defined groups.

All three patent applications concern dye transfer inhibition and the use of enhancers together with peroxidases as detergent additives or detergent compositions used in the detergent sector. The combination of these enhancers is limited to peroxidases. The use of mixtures containing peroxidases is also known from WO 92/18687. A special system of oxidases and of appropriate substrates such as hydrogen peroxide is described in German Unexamined Patent Application DE-42 31 761. German Unexamined Patent Application DE 19 18 729 concerns another special detergent system consisting of glucose and glucose oxidase or of starch, aminoglucosidase and glucose oxidase (GOD) and of added hydroxylamine or a hydroxylamine compound, wherein the hydroxylamine or the derivatives thereof serve to inhibit the catalase that is often present in GOD. Hydroxylamine and the derivatives thereof have definitely not been described as mediator additives.

Finally, WO 94/29425, DE 4445088.5 and WO 97/48786 concern multicomponent bleaching systems for use with detergents and which consist of oxidation catalysts and oxidants and of aliphatic, cycloaliphatic, heterocyclic or aromatic compounds containing NO-, NOH- or H-NR-OH groups.

All hitherto known "enzymatically enhanced" detergent-bleaching systems have the drawback that their cleaning and bleaching action is still unsatisfactory and that the mediators must be used in excessive amounts which may cause environmental and economic problems.

We have now found, quite surprisingly, that the enzyme component system (ECS) of the invention exceeds the performance of the aforesaid oxidoreductase-mediator systems and does not have the said drawbacks of the prior art, namely that the aforesaid objective can be reached by providing an enzyme component system (ECS) according to the invention which contains one or more lipases, preferably from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases, preferably from the group of amidases (3.5.1.4) (**system component 1**) which from one or more fatty acids present (preferably C₆- C₂₆ and particularly C₈ - C₁₆ fatty acids) (**system component 2**) and in the presence of an oxidant such as a peroxide, preferably H₂O₂ (**system component 3**) can produce peracids and in the presence of ketones as additional component (**system component 4**), for example, dioxiranes.

VIII) Use of the Enzyme Component System of the Invention in the Bleaching and/or Decolorization of Textile Fabrics

Enzymes are currently being used to an increasing extent in various applications in the textile industry. For example, the use of amylases in the desizing process is of great importance, because the use of strong acids, alkalies or oxidants is thereby avoided. Similarly, cellulases are used for biopolishing and biostoning, a process which is mostly employed together with conventional stone washing with pumice in the treatment of denim fabrics for jeans to remove the indigo dye. WO 94/29510, WO 96/18770, DE 196 12 194 A1 and DE 44 45 088 A1 describe enzymatic delignification processes which use enzymes together with mediators. In general, the disclosed mediators are compounds with the NO-, NOH- or HRNOH structure. These systems, of course, are restricted to use in pulp bleaching. Because the mechanisms underlying lignin-removing pulp bleaching, and this is the process involved here, are entirely different from those

underlying the decolorization, removal and/or "destruction" of denim dyes, particularly indigo dyes, in the jeans producing sector, it is entirely surprising that a number of substances of the said NO-, NOH- and HNROH types are also suitable for this application.

WO 97/06244 describes systems for the bleaching of pulp, for dye transfer inhibition and for bleaching stains when used with detergents, which systems employ enzymes (peroxidases, laccases) and enzyme-enhancing (hetero-)aromatic compounds, such as nitroso compounds etc.. In this case, as in patents WO 94/12619, WO 94/12620 and WO 94/12621, only the above-described use is intended. The mechanisms of stain decolorization in detergent bleaching or of dye transfer inhibition are entirely different from those underlying the decolorization, removal and/or "destruction" of indigo dyes, as, for example, in denim treatment. Hence, it is quite surprising that a number of substances of the said NO-, NOH- and HNROH-types are also suitable for this application.

Processes are known from said WO 94/12619, WO 94/12620 and WO 94/12621 in which the activity of peroxidase is increased by use of enhancers. Such enhancers are characterized in WO 94/12620 in terms of their half-life. According to WO 94/12621, enhancers are characterized by the formula $A=N-N=B$ where N means nitrogen and A and B are defined cyclic groups. According to WO 94/12621, enhancers are organic chemicals containing at least two aromatic rings of which at least one is substituted with defined groups. All three applications concern (as already stated) dye transfer inhibition and the use of enhancers together with peroxidases as detergent additives or detergent compositions for washing applications or in cellulose bleaching. The combinations of these enhancers are restricted to use with peroxidases.

Moreover, oxidoreductases, primarily laccases, but also peroxidases, are currently used, mainly for treating denim for jeans.

It is known from patent application WO 96/12846 that laccase and peroxidase + certain enhancers, mainly derivatives of phenothiazine or phenoxazine, are used in two application forms in the treatment of cellulose-containing fabrics, such as cotton, viscose, rayon (artificial silk), ramie, linen, Tencel, silk or mixtures thereof or mixtures of these fabrics with synthetic fibers, for example a mixture of cotton and spandex (stretch denim), but mainly denim fabrics (mainly for use in jeans).

On the one hand, the system (oxidoreductases + enhancers) is intended as a replacement for the conventional hypochlorite bleaching of denim, usually after a stone washing pretreatment, this enzymatic treatment providing only partial replacement of hypochlorite, because the desired bleaching effect cannot be attained.

On the other hand, the system can be used together with cellulase in stone washing in place of the usual mechanical treatment with pumice, and this represents an improvement over the "treatment with cellulase only".

The main drawbacks of the system described in WO 96/12846 are the following, among others:

- 1) To achieve the desired goal, laccase must be used in considerable amounts (about 10 international units [IU]/g of denim);
- 2) In some cases, optimum treatment requires 2-3 hours;

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- The general advantage of a laccase and/or oxidoreductase system with enzyme action-enhancing compounds (enhancers, mediators etc.) when used in the above-described treatment of textiles (for example, jeans fabrics) consists, in an improved system over the prior-art systems, in that fashion looks can be achieved, which is not possible with conventional hypochlorite bleaching.

The dyes normally used for jeans denim are vat dyes, such as indigo or indigo derivatives, for example thioindigo, as well as sulfur dyes. By use of such special enzymatic systems, it is possible (as a result of the high specificity of such systems), when a mixed dye system such as an indigo dye and a sulfur dye system is present, to decolorize only the indigo dye, while the sulfur dye is not oxidized. Depending on the enzyme action-enhancing compound used, this can produce almost any desired fabric color (for example, gray shades etc), which is often desirable.

An additional advantage is that the enzymatic treatment is substantially more gentle than bleaching with hypochlorite, and as a result fiber damage is reduced.

In the stone washing process, the ecological effect is of particular importance (in addition to the reduced fiber damage caused by enzymes) considering, for example, that this purely mechanical process produces about 1 kg of stone sludge per kg of jeans denim.

As can be seen from the prior art, for colored fabrics, in particular, the textile industry has a great need for alternative bleaching processes (alternatives to conventional hypochlorite bleaching) and/or treatment methods as alternatives to stone washing to achieve the bleached look, in the latter case also because of the environmental pollution problems.

The present invention has for an object to minimize or eliminate the drawbacks of the conventional processes: stone washing/bleaching after stone washing or general bleaching of dyed and/or undyed textile fabrics, particularly the pollution problems and fiber damage, as well as the drawbacks of the known oxidoreductase/enhancer systems (for example also NO-radical formation etc).

Entirely surprisingly, we have now found that the enzyme component System (ECS) of the invention exceeds the performance of the aforesaid oxidoreductase-mediator systems and that it does not exhibit the said drawbacks of the prior art.

In other words, according to the invention, the foregoing objective is reached by providing an enzyme component system (**ECS**) according to which contains one or more lipases, preferably from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases, preferably from the group of amidases (3.5.1.4) (**system component 1**) which from one or more fatty acids present (preferably C₆- C₂₆ and particularly C₈ – C₁₆ fatty acids) (**system component 2**) and in the presence of an oxidant such as a peroxide, preferably

Description of Individual System Components of the Enzyme Component System (ECS) of the Invention

Preferred are enzymes of group 3 (hydrolases), 3.1, 3.1.1, 3.1.2, 3.1.3, 3.1.4 and 3.1.7 according to the International Enzyme Nomenclature: Committee of the International Union of Biochemistry and Molecular Biology (Enzyme Nomenclature, Academic Press, Inc., 1992, pp. 306-337).

A) Carboxylate Ester Hydrolases (3.1.1)

- | | |
|----------|---------------------------------|
| 3.1.1.1 | Carboxylate esterase |
| 3.1.1.2 | Aryl esterase |
| 3.1.1.3 | Triacylglycerol lipase |
| 3.1.1.4 | Phospholipase A ₂ |
| 3.1.1.5 | Lysophospholipase |
| 3.1.1.6 | Acetyl esterase |
| 3.1.1.7 | Acetylcholine esterase |
| 3.1.1.8 | Choline esterase |
| 3.1.1.10 | Tropine esterase |
| 3.1.1.11 | Pectin esterase |
| 3.1.1.13 | Sterol esterase |
| 3.1.1.14 | Chlorophyllase |
| 3.1.1.15 | L-Arabinolactonase |
| 3.1.1.17 | Gluconolactonase |
| 3.1.1.19 | Uronolactonase |
| 3.1.1.20 | Tannase |
| 3.1.1.21 | Retenyl palmitate esterase |
| 3.1.1.22 | Hydroxybutyrate dimer hydrolase |
| 3.1.1.23 | Acylglycerol lipase |
| 3.1.1.24 | 3-Oxadipate enol lactonase |
| 3.1.1.25 | 1,4-Lactonase |
| 3.1.1.26 | Galactolipase |
| 3.1.1.27 | 4-Pyridoxolactonase |
| 3.1.1.28 | Acylcarnitine hydrolase |
| 3.1.1.30 | D-Arabinone lactonase |
| 3.1.1.31 | 6-Phosphogluconolactonase |
| 3.1.1.32 | Phospholipase A ₁ |
| 3.1.1.32 | 6-Acetylglucose deacetylase |
| 3.1.1.34 | Lipoprotein lipase |
| 3.1.1.35 | Dihydrocoumarin hydrolase |

- 3.1.1.36 Limonine D-ring lactonase
3.1.1.37 Steroid lactonase
3.1.1.38 Triacetate lactonase
3.1.1.39 Actinomycin lactonase
3.1.1.40 Orseilinate depside hydrolase
3.1.1.41 Cephalosporin C deacetylase
3.1.1.42 Chlorogenate hydrolase
3.1.1.43 α -Amino acid esterase
3.1.1.44 Methyloxaloacetate esterase
3.1.1.45 Carboxymethylenebutenolidase
3.1.1.46 Deoxylimonate A-ring lactonase
3.1.1.47 1-Alkyl-2-acetylglycerophosphocholine esterase
3.1.1.48 Fusarinine C ornithine esterase
3.1.1.49 Sinapine esterase
3.1.1.50 Wax ester hydrolase
3.1.1.51 Phorbol diester hydrolase
3.1.1.52 Phosphatidylinositol deacetylase
3.1.1.53 Sialate O-acetyl esterase
3.1.1.54 Acetoxybutynylbithiophene deacetylase
3.1.1.55 Acetylsalicylate deacetylase
3.1.1.56 Methylumbelliferyl acetate deacetylase
3.1.1.57 2-Pyrone-4,6-dicarboxygallate lactonase
3.1.1.58 N-Acetylgalactosaminoglycan deacetylase
3.1.1.59 Juvenile hormone esterase
3.1.1.60 Bis(2-ethylhexyl)phthalate esterase
3.1.1.61 Protein glutamate methylesterase
3.1.1.63 11-cis-Retynil palmitate hydrolase
3.1.1.64 all-trans-Retynil palmitate hydrolase
3.1.1.65 L-Rhamnono-1,4-lactonase
3.1.1.66 5-(3,4-diacetoxybutynyl)-2,2'-bithiophene deacetylase
3.1.1.67 Fatty acid ethyl ester synthase
3.1.1.68 Xylono-1,4-lactonase
3.1.1.69 N-Acetylglucosaminylphosphatidylinositol deacetylase
3.1.1.70 Cetaxate benzyl esterase

Also preferred are:

B) Thiol ester hydrolases (3.1.2)

- 3.1.2.6 Hydroxyacylglutathione hydrolase
3.1.2.7 Glutathione thiol esterase
3.1.2.12 S-Formylglutathione hydrolase
3.1.2.13 S-Succinylglutathione hydrolase
3.1.2.14 Oleoyl-(acyl carrier protein) hydrolase
3.1.2.15 Ubiquitin thiol esterase
3.1.2.16 (Citrate-(pro-3S)-lyase)thiol esterase.

Also preferred are:

C) Phosphoric Acid Monoester Hydrolases (Phosphatases) (3.1.3)

- | | |
|----------|--|
| 3.1.3.1 | Alkaline phosphatase |
| 3.1.3.2 | Acid phosphatase |
| 3.1.3.3 | Phosphoserine phosphatase |
| 3.1.3.4 | Phosphatidate phosphatase |
| 3.1.3.8 | 3-Phytase |
| 3.1.3.9 | Glucose-6-phosphatase |
| 3.1.3.10 | Glucose-1-phosphatase |
| 3.1.3.11 | Fructose biphosphatase |
| 3.1.3.12 | Trehalose phosphatase |
| 3.1.3.13 | Bisphosphoglycerate phosphatase |
| 3.1.3.14 | Methylphosphothioglycerate phosphatase |
| 3.1.3.15 | Histidinol phosphatase |
| 3.1.3.16 | Phosphoprotein phosphatase |
| 3.1.3.17 | (Phosphorylase) phosphatase |
| 3.1.3.18 | Phosphoglycolate phosphatase |
| 3.1.3.19 | Glycerol-2-phosphatase |
| 3.1.3.20 | Phosphoglycerate phosphatase |
| 3.1.3.21 | Glycerol-1-phosphatase |
| 3.1.3.22 | Mannitol-1-phosphatase |
| 3.1.3.23 | Sugar phosphatase |
| 3.1.3.24 | Sucrose phosphatase |
| 3.1.3.25 | Myoinositol-1 (or 4)-monophosphatase |
| 3.1.3.26 | 6-Phytase |
| 3.1.3.27 | Phosphatidylglycerophosphatase |
| 3.1.3.36 | Phosphatidylinositol biphosphatase |
| 3.1.3.37 | Sedoheptulose biphosphatase |
| 3.1.3.38 | 3-Phosphoglycerate phosphatase |
| 3.1.3.39 | Streptomycin-6-phosphatase |
| 3.1.3.40 | Guanidinodeoxy-scylo-inositol-4-phosphatase |
| 3.1.3.41 | 4-Nitrophenyl phosphatases |
| 3.1.3.42 | (Glycogen synthase-D) phosphatase |
| 3.1.3.43 | (Pyruvate dehydrogenase (lipoamide) phosphatase |
| 3.1.3.44 | 3-Deoxy-manno-octulosonate-8-phosphatase |
| 3.1.3.46 | Fructose-2,6-biphosphate-2-phosphatase |
| 3.1.3.48 | Protein-tyrosine phosphatase |
| 3.1.3.49 | (Pyruvate kinase) phosphatase |
| 3.1.3.50 | Sorbitol-6-phosphatase |
| 3.1.3.51 | Dolichyl phosphatase |
| 3.1.3.52 | 3-Methyl-2-oxobutanoate dehydrogenase) (lipoamide) phosphatase |
| 3.1.3.53 | Myosin light chain phosphatase |
| 3.1.3.54 | Fructose-2,6-bisphosphate-6-phosphatase |
| 3.1.3.55 | Caldesmon phosphatase |
| 3.1.3.56 | Inositol-1,4,5-triphosphate-B-phosphatase |
| 3.1.3.57 | Inositol-1,4-bisphosphate-1-phosphatase |
| 3.1.3.58 | Sugar terminal phosphatase |
| 3.1.3.59 | Alkylacetylgllycerophosphatase |
| 3.1.3.60 | Phosphoenolpyruvate phosphatase |
| 3.1.3.61 | Inositol-1,4,5-trisphosphate-1-phosphatase |
| 3.1.3.62 | Inositol-1,3,4,5-tetrakisphosphate-3-phosphatase |
| 3.1.3.63 | 2-Carboxy-D-arabinitol-1-phosphatase |

Also preferred are:

D) Phosphoric Acid Diester Hydrolases (3.1.4)

- 3.1.4.1 Phosphodiesterase I
- 3.1.4.2 Glycerophosphocholine phosphodiesterase
- 3.1.4.3 Phospholipase C
- 3.1.4.4 Phospholipase D
- 3.1.4.10 1-Phosphatidylinositol phosphodiesterase
- 3.1.4.11 1-Phosphatidylinositol-4,5-bisphosphate phosphodiesterase
- 3.1.4.12 Sphingomyelin phosphodiesterase
- 3.1.4.13 Serine-ethanolamine phosphate phosphodiesterase
- 3.1.4.14 (Acyl carrier protein) phosphodiesterase
- 3.1.4.36 1,2-Cyclic inositol phosphate phosphodiesterase
- 3.1.4.38 Glycerophosphocholine choline phosphodiesterase
- 3.1.4.39 Alkylglycerophosphoethanolamine phosphodiesterase
- 3.1.4.40 CMP-N-acetylneuraminate phosphodiesterase
- 3.1.4.41 Sphingomyelin phosphodiesterase D
- 3.1.4.42 Glycerol-1,2-cyclic phosphate-2-phosphodiesterase
- 3.1.4.43 Glycerophosphoinositol inositol phosphodiesterase
- 3.1.4.44 Glycerophosphoinositol glycerophosphodiesterase
- 3.1.4.45 N-acetylglucosamine-1-phosphodiesterase
- 3.1.4.46 Glycerophosphodiester phosphodiesterase
- 3.1.4.47 Variant surface glycoprotein phospholipase C
- 3.1.4.48 Dolichyl phosphate-glucose phosphodiesterase
- 3.1.4.49 Dolichyl phosphate-mannose phosphodiesterase
- 3.1.4.50 Glycoprotein phospholipase D
- 3.1.4.51 Glucose-1-phospho-D-mannosylglycoprotein phosphodiesterase.

Also preferred are:

E) Diphosphoric Acid Monoster Hydrolases (3.1.7)

- 3.1.7.1 Prenyl pyrophosphatase
- 3.1.7.3 Monoterpenyl pyrophosphatase

Particularly preferred among these are enzymes of group 3.1.1.3 lipases (triacylglycerol lipases, triglycerolacyl hydrolases) from organisms such as *Candida antarctica*, *Candida rugosa*, *Candida lipolytica*, *Candida cylindracea*, *Candida spec.*, *Geotrichum candidum*, *Humicola lanuginosa*, *Penicillium cambertii*, *Penicillium roquefortii*, *Aspergillus spec.*, *Mucor javanicus*, *Mucor mehei*, *Rhizopus arrhizus*, *Rhizopus niveus*, *Rhizopus delamar*, *Rhizopus spec.*, *Chromobacterium viscosum*, *Pseudomonas cepacia* and *Pseudomonas spec.* from wheat seedlings or pancreas (pig or other sources) or other sources.

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Enzymes splitting carbon/nitrogen (C/N) bonds (other than peptide bonds) can also be used (3.5).

This subclass includes enzymes capable of splitting amides, amidines and other C/N bonds. Particularly preferred are enzymes of class 3.5.1 which act on linear amides, of class 3.5.2 which act on cyclic amides, of class 3.5.3 which act on linear amidines, of class 5.3.1 which act on nitriles and of class 3.5.99 which act on other compounds.

Particularly preferred are enzymes of class 3.5.1. which act on linear amides:

- 3.5.1.1 Asparaginase
- 3.5.1.2 Glutaminase
- 3.5.1.3 ω -Amidase
- 3.5.1.4 Amidase
- 3.5.1.5 Urease
- 3.5.1.6 β -Ureidopropionase
- 3.5.1.7 Ureidosuccinase
- 3.5.1.8 Formylaspartate deformylase
- 3.5.1.9 Arylformamidase
- 3.5.1.10 Formyltetrahydrofolate deformylase
- 3.5.1.11 Penicillin amidase
- 3.5.1.12 Biotinidase
- 3.5.1.13 Arylacyl amidase
- 3.5.1.14 Aminoacylase
- 3.5.1.15 Aspartoacylase
- 3.5.1.16 Acetylornithine deacetylase
- 3.5.1.17 Acyl-lysine deacetylase
- 3.5.1.18 Nicotinamidase
- 3.5.1.20 Citrullinase
- 3.5.1.22 Pantothenase
- 3.5.1.30 5-Aminopentanamidase
- 3.5.1.31 Formylmethionine deformylase
- 3.5.1.32 Hippurate hydrolase
- 3.5.1.39 Alkylamidase
- 3.5.1.40 Acylagmatin amidase
- 3.5.1.41 Chitin deacetylase
- 3.5.1.42 Nicotinamide nucleotide amidase
- 3.5.1.49 Formamidase
- 3.5.1.50 Pentanamidase
- 3.5.1.55 Long-chain fatty acylglutamate deacylase
- 3.5.1.56 N,N-Dimethylformamidase
- 3.5.1.57 Tryptophanamidase
- 3.5.1.58 N-Benzyloxycarbonylglycine hydrolase

3.5.1.75 Urethanase

Also preferred are enzymes of class 3.5.2 which act on cyclic amides, such as:

- 3.5.2.1 Barbiturase
- 3.5.2.2 Dihydropyrimidase
- 3.5.2.3 Dihydroorotase
- 3.5.2.4 Carboxymethylhydantoinase
- 3.5.2.5 Allantoinase
- 3.5.2.6 β -Lactamase
- 3.5.2.10 Creatininase

Also particularly preferred are class 3.5.3 enzymes which act on linear amidines, such as:

- 3.5.3.1 Arginase
- 3.5.3.3 Creatinase
- 3.5.3.4 Allantoinase
- 3.5.3.6 Arginine deiminase
- 3.5.3.9 Allantoate deiminase
- 3.5.3.10 D-Arginase
- 3.5.3.14 Amidinoaspartase
- 3.5.3.15 Protein-arginine deiminase

Also particularly preferred are enzymes of class 3.5.4 which act on cyclic amidines, such as:

- 3.5.4.8 Aminoimidazolase
- 3.5.4.21 Creatinine deaminase

Preferred are also the enzymes of class 3.5.99 which act on other compounds, such as:

- 3.5.99.1 Riboflavinase
- 3.5.99.2 Thaminase

Particularly preferred enzymes are especially those of class 3.5.5. 1, nitrilase (3.5.5.2 - 3.5.5.6, other nitrilases).

Also particularly preferred are enzymes of class 3.5.1, here particularly those of class 3.5.1.4, amidases.

System Component 2 of the Enzyme Component System (ECS) of the Invention

Fatty acids which in the process according to the invention can be used as sources of peracids are, for example:

System Component 2 of the Enzyme Component System (ECS) of the Invention

Fatty acids which in the process according to the invention can be used as sources of peracids are, for example:

1) Saturated fatty acids

Butanoic acid	(butyric acid)
Pentanoic acid	(valeric acid)
Hexanoic acid	(caproic acid)
Heptanoic acid	(enanthic acid)
Octanoic acid	(caprylic acid)
Nonanoic acid	(pelargonic acid)
Decanoic acid	(capric acid)
Undecanoic acid	
Dodecanoic acid	(lauric acid)
Tridecanoic acid	
Tetradecanoic acid	(myristic acid)
Pentadecanoic acid	
Hexadecanoic acid	(palmitic acid)
Heptadecanoic acid	
Octadecanoic acid	(stearic acid)
Nonadecanoic acid	
Eicosanoic acid	(arachic acid)
Heneicosanoic acid	
Docosanoic acid	(behenic acid)
Tricosanoic acid	
Tetracosanoic acid	(lignoceric acid)
Pentacosanoic acid	
Hexacosanoic acid	(cerotic acid)
Octacosanoic acid	
Triacosanoic acid	(melissic acid)

2) Unsaturated fatty acids

10-Undecenoic acid	
9-cis-Dodecenoic acid	(lauroleic acid)
9-cis-Tetradecenoic acid	(myristoleic acid)
9-cis-Hexadecenoic acid	(palmitleic acid)
6-cis-Octadecenoic acid	(petroselinic acid)
6-trans-Octadecenoic acid	(petroselinic acid)
9-cis-Octadecenoic acid	(oleic acid)
9-trans-Octadecenoic acid	(elaidic acid)
9-cis, 12 cis-Octadecadienoic acid	(linoleic acid)
9-trans, 12-trans-Octadecadienoic acid	(linoleic acid)
9-cis, 12-cis, 15-cis-Octadecatrienoic acid	(linolenic acid)
9-trans, 11-trans, 13-trans-Octadecatrienoic acid	(α -eleostearic acid)
9-cis, 11-trans, 13-trans-Octadecatrienoic acid	(β -eleostearic acid)

4,8,12,15,19-Docosapentaenoic acid

(clupanodonic acid)

3) Polyunsaturated fatty acids

9,12-Octadecadienoic acid

(linoleic acid)

9,12,15-Octadecatrienoic acid

(linolenic acid)

5,9,12-Octadecatrienoic acid

9,11,13-Octadecatrienoic acid

(eleostearic acid)

9,11,13,15-Octadecatetraenoic acid

(parinaric acid)

5,11,14-Icosatrienoic acid

5,8,11,14-Icosatetraenoic acid

(arachidic acid)

4,8,12,15,18-Icosapentaenoic acid

4,8,12,15,19-Docosapentaenoic acid

(clupanodonic acid)

4,8,12,15,18,21-Tetracosahexaenoic acid

(nisinic acid)

Particularly preferred are tetradecanoic acid (myristic acid) and dodecanoic acid (lauric acid).

System Component 3 (Oxidants: Peroxides or Per Compounds) of the Enzyme Component System (ECS) of the Invention

Preferred oxidants in the enzyme component system of the invention are hydrogen peroxide (H_2O_2), organic peroxides and per-compounds such as perborates, persulfates, percarbonates, perphosphates, percarbamides, perchlorates etc.

Preferred organic peroxides are, for example:

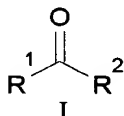
3-chloroperoxybenzoic acid, monoperoxyphthalic acid Mg salt, di-tert.butyl peroxide, cumene hydroperoxide, lauroyl peroxide, chloroperoxybenzoic acid, dicumyl peroxide, methyl ethyl ketone peroxide, benzoyl peroxide, diperoxidododecandionic acid Na salt etc..

Besides lipase-catalyzed peracid formation, combinations of bleach activators, such as TAED (tetraacetythylenediamine), TAGU (tetraacetyl glycoluril) and iso-NOBS (sodium p-isononanoyl-oxybenzenesulfonate) and the like, which are also used in detergents, together with per-compounds such as perborates, percarbonates etc. can serve as additional sources of peracid generation.

The abovesaid per-compounds, as well as, for example, glucose + GOD, can be used as systems generating H_2O_2 for the corresponding lipase action. Substances such as nitrilamines or dicyandiamines or metal ions, e.g. Mo^{6+} , V^{6+} and W^{6+} can be used together with a peroxide, for example H_2O_2 .

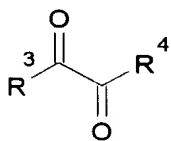
System Component 4 (Ketones) of the Enzyme Component System (ECS) of the Invention

Particularly preferred are carbonyl compounds of general formula I:

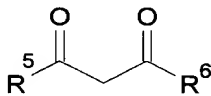


The R^1 and R^2 groups can be equal or different and denote aliphatic or aromatic groups. Moreover, the R^1 and R^2 groups can form a ring containing besides carbon also heteroatoms such as nitrogen, oxygen and sulfur.

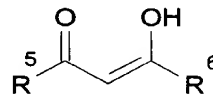
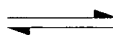
Particularly preferred are 1,2-diketones (formula II), 1,3-diketones (formula III), polyketones (polyketides) and the tautomeric enols (formula IV):



II



III



IV

wherein the R^3 to R^6 groups, once again, can be equal or different and denote aliphatic or aromatic groups. Moreover, groups R^3 and R^4 and groups R^5 and R^6 , together, can form a ring containing besides carbon also heteroatoms such as nitrogen, oxygen or sulfur. The possibility of tautomerization or formation of a resonance hybrid is particularly important in this case.

Besides general carbonyl compounds, particularly preferred are ketones, such as, in general hydroxyketones, α,β -unsaturated ketones, oxycarboxylic acids, quinones and halogenated ketones.

Particularly preferred among these are the following:

Acetone, methyl ethyl ketone, diethyl ketone, methyl n-butyl ketone, methyl isobutyl ketone, cyclohexanone, cyclopentanone, 2-methylcyclohexanone, 3-methylcyclohexanone, 4-methylcyclohexanone, dihydroxyacetone, diacetyl monohydrazone, diacetyl dihydrazone, acetophenone, p-hydroxyacetophenone, 1-phenyl-3-butanone, 3-pentanone, 4-heptanone, 2-nonanone, cycloheptanone, cyclooctanone, cyclodecanone, cyclododecanone, dimethyl ketone, ethyl propyl ketone, methyl amyl ketone, acetylacetone, pinacolone, methyl isopropyl ketone, methyl isoamyl ketone, ethyl amyl ketone diisopropyl ketone, diisobutyl ketone, methyl vinyl ketone, methyl isopropenyl ketone, mesityl oxide, isophorone, hydroxyacetone, methoxyacetone, 2,3-pentanedione, 2,3-hexanedione, phenylacetone, propiophenone, benzophenone, benzoin, benzil, 4,4'-dimethoxybenzil, 4'-methoxyacetophenone, 3'-methoxyacetophenone, O-ethylbenzoin, (2-methoxyphenyl)acetone, (4-methoxyphenyl)acetone, methoxy-2-propanone, glyoxylic acid, benzyl glyoxylate, benzylacetone, methyl benzyl ketone, methylcyclohexyl ketone, 2-decanone, dicyclohexyl ketone, 3,3-dimethyl-2-butanone, methyl isobutyl ketone, methyl isopropyl ketone, 2-methyl-3-heptanone, 5-methyl-3-heptanone, 6-methyl-5-hepten-2-one, 5-methyl-2-hexanone, 3-nonanone, 5-nonanone, 2-octanone, 3-octanone, 2-undecanone, 1,3-dichloroacetone, 1-hydroxy-2-butanone, 3-hydroxy-2-butanone, 4-hydroxy-4-methyl-2-pentanone, 2-(1S)-adamanantone, anthrone, bicyclo(3.2.0)hept-2-en-6-one, cis-bicyclo(3.3.0)octan-3,7-dione, (1S)-(-)-camphor, p-chloranil, cyclobutanone, 1,3-cyclohexanedione, 1,4-cyclohexanedione monoethylene ketal, dibenzosuberone, ethyl 4-oxocyclohexanecarboxylate, 9-fluorenone, 1,3-indandione, methylcyclohexanone, phenylcyclohexanone, 4-propylcyclohexanone, 1,2,3,4-tetrahydro-1-naphthalenone, 1,2,3,4-tetrahydro-2-naphthalenone, 3,3,5-trimethylcyclohexanone, 3-acetoxy-2-cyclohexen-1-one, benzylideneacetone, (R)-(-)-carvone, (S)-(-)-carvone, curcumin, 2-cyclohexen-1-one, 2,3-diphenyl-2-cyclopropen-1-one, 2-hydroxy-3-methyl-2-cyclopentene-1-one, isophorone, α -ionone, β -ionone, 3-methoxy-2-cyclohexen-1-one, 3-methyl-2-cyclopenten-1-one, 3-methyl-3-penten-2-one, (R)-(+)-pulegone, tetraphenyl-2,4-cyclopentadien-1-one, 2,6,6-trimethyl-2-cyclohexen-1,4-dione, 2-acetylbenzoic acid, 1-acetylnaphthalene, 2-acetylnaphthalene, 3'-aminoacetophenone, 4'-aminoacetophenone, 4'-cyclohexylacetophenone, 3',4'-diacetoxyacetophenone, diacetylbenzene, 2',4'-dihydroxyacetophenone, 2',5'-dihydroxyacetophenone, 2',6'-dihydroxyacetophenone, 3,4-dimethoxyacetophenone, 2'-hydroxyacetophenone, 4'-hydroxyacetophenone, 3'-methoxyacetophenone, 4'-methoxyacetophenone, 2'-methylacetophenone, 4'-methylacetophenone, 2'-nitroacetophenone, 3'-nitroacetophenone, 4'-phenylacetophenone, 3',4',5'-trimethoxyacetophenone, 4'-aminopropiophenone, benzoylacetone, benzoylpropionic acid, benzylideneacetophenone, cyclohexyl phenyl ketone, desoxybenzoin, 4',4'-dimethoxybenzil, 1,3-diphenyl-1,3-propanedione, ethylbenzoyl acetate, ethyl phenylglyoxylate, 4'-hydroxypropiophenone, 1,3-indandione, 1-indanone, isopropyl phenyl ketone, 6-methoxy-1,2,3,4-tetrahydronaphthalen-1-one, methylphenyl glyoxylate, phenylglyoxylonitrile, 1-phenyl-1,2-propanedione 2-oxime, valerophenone, 2-acetyl- γ -butyrolactone, 2-acetylpyrrole, 1-benzylpiperidin-4-one, dehydroacetic acid, 3,4-dihydro-4,4-dimethyl-2H-pyran-2-one, 1,4-dihydro-4-pyridinone, N-ethoxycarbonyl-4-piperidinone, 2-methyl furyl ketone, 5-hydroxy-2-hydroxymethyl-4H-

pyran-4-one, 3-hydroxy-2-methyl-4-pyranone, 3-indolyl methyl ketone, isatin, 1-methyl-4-piperidinone, methyl 2-pyridyl ketone, methyl 3-pyridyl ketone, methyl 4-pyridyl ketone, methyl 2-thienyl ketone, phenyl 2-pyridyl ketone, phenyl 4-pyridyl ketone, tetrahydrofuran-2,4-dione, tetrahydro-4H-pyran-4-one, 2,2,6,6-tetramethyl-4-piperidone, xanthone, acenaphthene quinone, pyruvic acid, (1 R)-(-)-camphor quinone, (1S)-(+)-camphor quinone, 3,5-ditert.butyl-o-benzoquinone, 1,2-dihydroxy-3,4-cyclobutendione, ethyl (2-amino-4-thiazolyl)glyoxylate, ethyl pyruvate, 2,3-hexanedione, 3,4-hexanedione, 3-methyl-2-oxobutyric acid, 3-methyl-2-oxovaleric acid, 4-methyl-2-oxovaleric acid, 2-oxobutyric acid, 2,3-pentandione, 9,10-phenanthrene quinone, acetoacetanilide, 2-acetyl- γ -butyrolactone, 2-acetylcyclopentanone, allyl acetoacetate, benzoylacetone, tert.butyl acetoacetate, 1,3-cyclopentanedione, diethyl 3-oxoglutarate, dimethyl acetylsuccinate, dimethyl 3-oxoglutarate, 1,3-diphenyl-1,3-propanedione, ethyl acetoacetate, ethyl benzoylacetate, ethyl butyrylacetate, ethyl 2-oxocyclohexanecarboxylate, ethyl 2-phenylacetoacetate, methyl acetoacetate, 2-methyl-1,3-cyclohexanedione, 2-methyl-1,3-cyclopentanedione, methyl isobutyrylacetate, methyl 3-oxopentanoate, methyl pivaloylacetate, 3-oxoglutaric acid, tetrahydrofuran-2,4-dione, 2,2,6,6-tetramethyl-3,5-heptanedione, 3-benzoylpropionic acid, 1,4-cyclohexanedione, dimethyl acetylsuccinate, ethyl levulinate, 2-aminoanthraquinone, anthraquinone, p-benzoquinone, 1,4-dihydroxyanthraquinone, 1,8-dihydroxyanthraquinone, 2-ethylanthraquinone, methyl-p-benzoquinone, 1,4-naphthoquinone, tetramethyl-p-benzoquinone, 2,2-dimethyl-1,3-dioxan-4,6-dione, 2-benzoylbenzoic acid, 3-benzoylpropionic acid, 5,6-dimethoxyphthalaldehydic acid, levulinic acid, methyl trans-4-oxo-2-pentenoate, phthalaldehydic acid, terephthalaldehydic acid, dibutyl maleate, dibutyl succinate, dibutyl phthalate, dicyclohexyl phthalate, diethyl acetamidomalonate, diethyl adipate, diethyl benzylmalonate, diethyl butylmalonate, diethylethoxymethylmalonate, diethyl ethylmalonate, diethyl fumarate, diethyl glutarate, diethyl isopropylidenemalonate, diethyl maleate, diethyl malonate, diethyl methylmalonate, diethyl oxalate, diethyl 3-oxoglutarate, diethyl phenylmalonate, diethyl phthalate, diethyl pimelate, diethyl sebacate, diethyl suberate, diethyl succinate, diisobutyl phthalate, dimethyl acetylene-dicarboxylate, dimethyl acetylsuccinate, dimethyl adipate, dimethyl 2-aminoterephthalate, dimethyl fumarate, dimethyl glutaconate, dimethyl glutarate, dimethyl isophthalate, dimethyl malonate, dimethylmethoxymalonate, dimethyl methylenesuccinate, dimethyl oxalate, dimethyl 3-oxoglutarate, dimethyl phthalate, dimethyl succinate, dimethyl terephthalate, ethylene glycol diacetate, ethylene glycol dimethacrylate, monoethyl fumarate, monomethyl malonate, monoethyl adipate, monomethyl phthalate, monomethyl pimelate, monomethyl terephthalate, 1,2-propylene glycol diacetate, triethyl methanetricarboxylate, trimethyl 1,2,3-propanetricarboxylate, 3-acetoxy-2-cyclohexen-1-one, allyl acetoacetate, allyl cyanoacetate, benzyl acetoacetate, tert.butyl acetoacetate, butyl cyanoacetate, chlorogenic acid hemihydrate, coumarin-3-carboxylic acid, diethyl ethoxycarbonylmethanephosphonate, dodecyl gallate, dodecyl 3,4,5-trihydroxybenzoate, (2,3-epoxypropyl) methacrylate, (2-ethoxyethyl) acetate, ethyl acetamidocyanoacetate, ethyl 2-aminobenzoate, ethyl 3-aminopyrazol-4-carboxylate, ethyl benzoxylacetate, ethyl butyrylacetate, ethyl cyanoacetate, ethyl 2-cyano-3-ethoxyacrylate, ethyl cyanoformate, ethyl 2-cyanopropionate, ethyl 3,3-diethoxypropionate, ethyl 1,3-dithian-2-carboxylate, ethyl 2-ethoxyacetate, ethyl 2-furancarboxylate, ethyl levulinate, ethyl mandelate, ethyl gallate, ethyl 2-methyl lactate, ethyl 4-nitrocinnamate, ethyl oxamate, ethyl 2-

oxocyclohexanecarboxylate, ethyl 4-oxocyclohexane- carboxylate, ethyl 5-oxohexanoate, ethyl 2-phenylacetoacetate, ethyl 4-piperidinecarboxylate, ethyl 2-pyridinecarboxylate, ethyl 3-pyridinecarboxylate, ethyl 4-pyridinecarboxylate, ethyl thioglycolate, ethyl 3,4,5-trihydroxybenzoate, 2-hydroxyethyl methacrylate, 2-hydroxypropyl methacrylate, 3-indole acetate, 2-methoxyethyl acetate, 1-methoxy-2-propyl acetate, methyl 2- aminobenzoate, methyl 3-aminocrotonate, methyl cyanoacetate, methyl 4-cyanobenzoate, methyl 4-formylbenzoate, methyl 2-furancarboxylate, methyl isobutyrylacetate, methyl methoxyacetate, methyl 2-methoxybenzoate, methyl 3-oxopentanoate, methyl phenylglyoxylate, methyl phenyl- sulfinylacetate, methyl pivaloylacetate, methyl 3-pyridinecarboxylate, 5-nitrofurfurylidene diacetate, propyl gallate, propyl 3,4,5-trihydroxybenzoate, methyl 3-methylthiopropionate, acetamide, acetanilide, benzamide, benzanilide, N,N-diethylacetamide, N,N-dimethylformamide, N,N-diethyl-3-methyl- benzamide, diethyltoluamide, N,N-dimethylacetamide, N,N-diphenylacetamide, N-methylformamide, N-methylformanilide, N-acetylthiourea, adipic acid diamide, 2-aminobenzamide, 4-aminobenzamide, succinic acid diamide, malonic acid diamide, N,N'-methylene diacrylamide, oxalic acid diamide, pyrazine-2-carboxamide, pyridine-4-carboxamide, N,N,N',N'-tetramethylsuccinic acid diamide, N,N,N',N'-tetramethylglutaric acid diamide, acetoacetanilide, benzohydroxamic acid, cyanoacetamide, 2-ethoxybenzamide, diethyl acetamidomalonate, ethyl acetamidocyanoacetate, ethyl oxamate, hippuric acid Na salt, N-(hydroxymethyl)acrylamide, L-(-)-lactamide, 2'-nitroacetanilide, 3'- nitroacetanilide, 4'-nitroacetanilide, paracetamol, piperine, salicylanilide, 2-acetyl- γ -butyrolactone, γ -butyrolactone, ϵ -caprolactone, dihydrocoumarin, 4-hydroxycoumarin, 2-(5H)-furanone, 2,5-dihydro-5-methoxy-2-furanone, phthalide, tetrahydrofuran-2,4-dione, 2,2,6-trimethyl-1,3-dioxin-4-one, γ - valerolactone, 4-amino-1,3-dimethyluracil, barbituric acid, O-benzyloxycarbonyl-N-hydroxysuccinimide, succinimide, 3,6-dimethylpiperazin-2,5-dione, 5,5-diphenylhydantoin, ethyl 1,3-dioxoisindoline-2-carboxylate, 9-fluorenylmethylsuccinimidyl carbonate, hydantoin, maleimide, 3-methyl-1- phenyl-2-pyrazolin-5-one, 1-methyl-2-pyrrolidone, methyluracil, 6-methyluracil, oxindole, phenytoin, 1-(2H)-phthalazinone, phthalimide, 2,5-piperazinedione, 2-piperidinone, 2-pyrrolidone, rhodanine, saccharin, 1,2,3,6-tetrahydrophthalimide, 1,2,3,4-tetrahydro-6,7-dimethoxyquinazolin-2,4-dione, 1,5,5-trimethyl-hydantoin, 1-vinyl-2-pyrrolidone, ditert.butyl dicarbonate, diethyl carbonate, dimethyl carbonate, dimethyl dicarbonate, diphenyl carbonate, 4,5-diphenyl-1,3-dioxol-2-one, 4,6- diphenylthieno-(3,4-d)-1,3-dioxo[2-one 5,5-dioxide, ethylene carbonate, magnesium methoxide methyl carbonate, monomethyl carbonate Na salt, propenyl carbonate, N-allylurea, azodicarbonamide, N-benzylurea, biuret, 1,1'-carbonyldiimidazol, N,N-dimethylurea, N-ethylurea, N-formylurea, urea, N-methylurea, N-phenylurea, 4-phenylsemicarbazide, tetramethylurea, semicarbazide hydrochloride, diethyl azodicarboxylate, methyl carbamate, 1-(4-methoxyphenyl)-2-(2- methoxyphenoxy)ethanone and 1-(4-methoxyphenyl)-2-(2-methoxyphenoxy)ethanol.

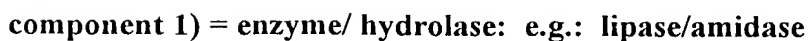
Also preferred are anhydrides, such as the following:

Benzoic anhydride, benzene-1,2,4,5-tetracarboxylic acid-1,2,4,5-dianhydride, 3,3',4,4'-benzophenonetetracarboxylic anhydride, succinic anhydride, butyric anhydride, crotonic anhydride, cis-1,2-cyclo- hexanedicarboxylic anhydride, ditert.butyl dicarbonate,

Particularly preferred are benzophenones such as the following:

Figure 1 shows schematically a possible reaction cycle involving all components.

(D = dioxirane) **(C = ketone)** **B: e.g.: per-fatty acid**
 (R 1 = 00H)



component 2) = fatty acid (A)

component 3) = oxidation agent, e.g.: H_2O_2 (Oxi)

B) = e.g.: per-fatty acid

D) = dioxirane

A more detailed description of the enzyme component system (ECS) of the invention in terms of various applications follows:

1) Use in Wood Pulp Bleaching

One of the components of the enzyme component system (ECS) of the invention used is an enzyme, preferably lipase from, for example, *Humicola lanuginosa*, used at a concentration of 0.05 to 5 mg per gram of wood pulp, preferably from 0.05 to 2 mg of enzyme per gram of wood pulp (which corresponds to about 250 to 10,000 IU per gram of wood pulp) (1 IU hydrolyzes 1 μ equivalent of a triglyceride fatty acid in 1 hour at pH 7.7 and 37 °C).

Preferably, the delignification (bleaching) with the enzyme component system of the invention is carried out in the presence of oxygen or air at atmospheric pressure or at a slight positive O₂ pressure and at pH from 2 to 11, preferably at pH 3-9, at a temperature of 20 to 95 °C, preferably 40-95 °C and a pulp consistency of 0.5 to 40%. An unusual and surprising finding concerning the use of enzymes for wood pulp bleaching is that when the enzyme component system of the invention is used, the consistency of the material can be increased and the kappa value thus markedly reduced. For economic reasons, the process according to the invention is carried out at a pulp consistency from 4 to 35% and particularly from 4 to 15%.

Another component is the oxidant, preferably H₂O₂, which is used at a concentration from 0.05 to 20 mg/g of wood pulp (100% basis) and preferably from 0.05 to 10 mg/g of wood pulp.

Another component consists of one or more fatty acids, preferably C₆ to C₂₆, and particularly C₆ to C₁₆ fatty acids and more particularly tetradecanoic or dodecanoic acid at a concentration from 0.05 to 20 mg/g of wood pulp and preferably at a concentration from 0.05 to 10 mg/g of wood pulp.

Another component are the ketones, preferably, for example, benzophenone at a concentration from 0.05 to 20 mg/g of wood pulp and preferably at a concentration from 0.05 to 10 mg/g of wood pulp.

When the enzyme component system of the invention is used, for example, in a process for treating lignin, the chosen components are mixed with an aqueous suspension of the lignin-containing pulp simultaneously or in any order. The reaction is preferably started by adding the oxidant or the enzyme.

Besides the abovesaid main components of the enzyme component system (ECS) of the invention, namely the enzymes (lipases/amidases), the oxidant, the fatty acids and the ketones, the bleaching system can also contain phenolic and/or nonphenolic compounds with one or more benzene rings which are capable of improving "oxidation transfer"

The chelates present in the solution can also serve as mimicking substances for certain oxido- reductases, such as the laccases (copper complexes) or for the lignin or manganese peroxidases (heme complexes). By mimicking substances are meant substances simulating the prosthetic groups of (in the present case) oxidoreductases and, for example, capable of catalyzing oxidation reactions.

Finally, it is also possible to use detergents. These include nonionic, anionic, cationic and amphoteric surfactants. Detergents improve the penetration of the enzymes and other components into the fibers.

Other proteins that can be added are proteases such as pepsin, bromelain, papain etc.

Other suitable protective colloids are amino acids, monosaccharides, oligosaccharides, polyethylene glycol [PEG] types of a wide range of molecular weights, polyethylene oxides, polyethyleneimines and polydimethylsiloxanes.

It is also possible to add to the enzyme component system of the invention substances capable of increasing the hydrophobicity of the reaction mixture, thus bringing about the swelling of the lignin and the fibers and which makes them more susceptible to attack. Such substances are, for example, glycols, such as propylene glycol and ethylene glycol, glycol ethers such as ethylene glycol dimethyl ether etc., and solvents, for example, alcohols such as methanol, ethanol, butanol, amyl alcohol, cyclohexanol, benzyl alcohol and chlorohydrin, phenols such as phenol, methylphenols and methoxyphenols, aldehydes such as formaldehyde and chloral, mercaptans such as butyl mercaptan, benzyl mercaptan and thioglycolic acid, organic acids such as formic, acetic and chloroacetic acid, amines such as ammonia and hydrazine, hydrotropic solvents, for example concentrated solutions of sodium benzoate, other substances such as benzenes, pyridines, dioxane, ethyl acetate, and other basic solvents such as $\text{OH}^-/\text{H}_2\text{O}$ or $\text{OH}^-/\text{alcohol}$ etc..

The process according to the invention can be used not only for the delignification (bleaching) of sulfate, sulfite, organosolv or other wood pulps or lignins, but also for the preparation of wood pulp in general, whether from wood or annual plants, when it is desired to carry out the defibrillation by the usual cooking (digestion) process (possibly combined with mechanical processing or pressure), namely by very gentle digestion, up to kappa numbers in the range from about 50 - 120 kappa.

In the bleaching as in the preparation of wood pulps, the treatment with the enzyme component system (ECS) of the invention can be applied once or several times, either before and/or after the washing and extraction of the treated material with NaOH etc., or without these intermediate steps, but also before and/or after pretreatment or post-treatment steps, such as acid washing, Q-steps, alkaline leaching or bleaching steps such as peroxide bleaching, O₂-enhanced peroxide steps, pressurized peroxide steps, O₂-delignification, Cl₂-bleaching, ClO₂-bleaching, Cl₂/ClO₂-bleaching, peracid bleaching, peracid-enhanced O₂/peroxide bleaching, ozone bleaching, dioxirane bleaching, reductive bleaching steps, other treatments such as swelling steps, sulfonations, NO/NO₂ treatments, nitrosylsulfuric acid treatment, enzyme treatments, for example treatments with hydrolases, such as cellulases and/or hemicellulases (for example, xylanase, mannanase etc) and/or amylases and/or pectinases and/or proteinases and/or lipases and/or amidases and/or oxidoreductases such as, for example, laccases and/or peroxidases etc., or several combined treatments.

The invention will be further illustrated by way of the following examples:

Enzymatic Bleaching of O₂-delignified Softwood (Sulfate Pulp)

A) To 20 mL of tap water were added 1 mg of tetradecanoic acid, 5 mg of benzophenone and 2.5 mg of H₂O₂ (30%) with agitation. The pH was adjusted with sulfuric acid and/or sodium hydroxide solution so that, after addition of the wood pulp and the enzyme, the pH was 7.5.

B) To 5 mL of tap water was added 5 mg of lipase from *Humicola lanuginosa* (about 25,000 IU).

Solutions A and B were combined and diluted to 33 mL. After addition of the wood pulp, the material was mixed in a dough mixer for 2 minutes. The material was then transferred to a reaction vessel preheated to 45 °C and was allowed to incubate 1-4 hours under atmospheric pressure.

The material was washed over a nylon screen (30 μm) and extracted for one hour at 60 $^{\circ}\text{C}$, 2% consistency and using 8% NaOH per gram of wood pulp. The material was again washed after which the kappa number was determined. For results see Table 1.

Enzymatic Bleaching of O₂-delignified Softwood (Sulfate Pulp)

5 g, absolutely dry basis, of wood pulp (O₂-delignified softwood), pulp consistency 30% (about 17 g moist) was added to solutions prepared as follows:

A) To 20 mL of tap water were added 1 mg of tetradecanoic acid, 5 mg of acetone and 2.5 mg of H₂O₂ (30%) with agitation. The pH was adjusted with sulfuric acid and/or sodium hydroxide solution so that, after addition of the wood pulp and the enzyme, the pH was 7.5.

B) To 5 mL of tap water was added 5 mg of lipase from *Humicola lanuginosa* (about 25,000 IU).

Solutions A and B were combined and diluted to 33 mL. After addition of the wood pulp, the material was mixed in a dough mixer for 2 minutes. The material was then

transferred to a reaction vessel preheated to 45 °C and was allowed to incubate 1-4 hours at atmospheric pressure.

The material was washed over a nylon screen (30 µm) and extracted for one hour at 60 °C, 2% pulp consistency and using 8% NaOH per gram of wood pulp. The material was again washed, after which the kappa number was determined. For results see Table 1.

EXAMPLE 1b (+ H₂O₂, with Nitrilamine as Activator)

Enzymatic Bleaching of O₂-delignified Softwood (Sulfate Pulp)

5 g, absolutely dry basis, of wood pulp (O₂-delignified softwood), pulp consistency 30% (about 17 g moist) was added to solutions prepared as follows:

A) To 20 mL of tap water were added 1 mg of tetradecanoic acid, 5 mg of acetone, 2.5 mg of H₂O₂ (30%) and 0.5 mg of nitrilamine with agitation. The pH was adjusted with sulfuric acid and/or sodium hydroxide solution so that, after addition of the wood pulp and the enzyme, the pH was 7.5.

B) To 5 mL of tap water was added 5 mg of lipase from *Humicola lanuginosa* (about 25,000 IU).

Solutions A and B were combined and diluted to 33 mL. After addition of the wood pulp, the material was mixed in a dough mixer for 2 minutes. The material was then transferred to a reaction vessel preheated to 45 °C and was allowed to incubate 1-4 hours at atmospheric pressure.

The material was washed over a nylon screen (30 µm) and extracted for one hour at 60 °C, 2 % pulp consistency and using 8% NaOH per gram of wood pulp. The material was again washed, after which the kappa number was determined. For results see Table 1.

EXAMPLE 2

Enzymatic Bleaching of O₂-delignified Hardwood (Sulfate Pulp)

5 g, absolutely dry basis, of wood pulp (O₂-delignified hardwood), pulp consistency 30% (about 17 g moist) was added to solutions prepared as follows:

A) To 20 mL of tap water were added 1 mg of tetradecanoic acid, 5 mg of benzophenone and 2.5 mg of H₂O₂ (30%) with agitation. The pH was adjusted with sulfuric acid and/or sodium hydroxide solution so that, after addition of the wood pulp and the enzyme, the pH was 7.5.

B) To 5 mL of tap water was added 5 mg of lipase from *Humicola lanuginosa* (about 25,000 IU).

Solutions A and B were combined and diluted to 33 mL. After addition of the wood pulp, the material was mixed in a dough mixer for 2 minutes. The material was then transferred to a reaction vessel preheated to 45 °C and was allowed to incubate 1-4 hours at atmospheric pressure.

The material was washed over a nylon screen (30 µm) and extracted for one hour at 60 °C, 2% pulp consistency and using 8% NaOH per gram of wood pulp. The material was again washed after which the kappa number was determined. For results see Table 1.

EXAMPLE 2a

5 g, absolutely dry basis, of wood pulp (O₂-delignified hardwood), pulp consistency 30% (about 17 g moist) was added to solutions prepared as follows:

A) To 20 mL of tap water were added 1 mg of tetradecanoic acid, 5 mg of acetone and 2.5 mg of H₂O₂ (30%) with agitation, The pH was adjusted with sulfuric acid and/or sodium hydroxide solution so that, after addition of the wood pulp and the enzyme, the pH was 7.5.

B) To 5 mL of tap water was added 5 mg of lipase from *Humicola lanuginosa* (about 25,000 IU).

Solutions A and B were combined and diluted to 33 mL. After addition of the wood pulp, the material was mixed in a dough mixer for 2 minutes. The material was then transferred to a reaction vessel preheated to 45 °C and was allowed to incubate 1-4 hours at atmospheric pressure.

The material was washed over a nylon screen (30 µm) and extracted for one hour at 60 °C, 2% pulp consistency and using 8% NaOH per gram of wood pulp. The material was again washed, after which the kappa number was determined. For results see Table 1.

EXAMPLE 3

Enzymatic Bleaching of O₂-delignified Softwood (Sulfate Pulp)

5 g, absolutely dry basis, of wood pulp (O₂-delignified softwood), pulp consistency 30% (about 17 g moist) was added to solutions prepared as follows.

A) To 20 mL of tap water were added 1 mg of tetradecanoic acid, 5 mg of benzophenone and 2.5 mg of H₂O₂ (30%) with agitation. The pH was adjusted with sulfuric acid and/or sodium hydroxide solution so that, after addition of the wood pulp and the enzyme, the pH was 7.5.

B) To 5 mL of tap water was added 200 IU of amidase from *Pseudomonas aeruginosa* (Sigma A 6691) (1 IU = conversion of 1 µmole of acetamide and hydroxylamine to acetohydroxamic acid and NH₃ per minute at pH 7.2 and 37 °C).

Solutions A and B were combined and diluted to 33 mL. After addition of the wood pulp, the material was mixed in a dough mixer for 2 minutes. The material was then transferred to a reaction vessel preheated to 45 °C and was allowed to incubate 1-4 hours at atmospheric pressure.

The material was washed over a nylon screen (30 µm) and extracted for one hour at 60 °C, 2% pulp consistency and using 8% NaOH per gram of wood pulp. The material was again washed, after which the kappa number was determined. For results see Table 1.

TABLE 1

Wood pulp	% Delignification (before extraction)	% Delignification (after extraction)
a) Softwood (untreated)	----	5.8%
b) Softwood (lipase-treated)	19% <u>17.5%</u>	32.0% <u>31%*</u> <u>35%**</u>
c) Hardwood (untreated)	-----	6.5%
d) Hardwood (lipase-treated)	21% <u>18%*</u>	33% <u>28%*</u>
e) Softwood (amidase-treated)	15.5%	23%
f) Comparative example: laccase + HOBT 5 kg/ton of wood pulp, other conditions as in WO 96/18770 (pulp a/b)	17.5%	22%

*Underlined values were obtained with acetone as the ketone.

**Value obtained with added nitrilamine.

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Because in this application the polymerization of lignin or lignin constituents contained in the wastewater is desired rather than lignin degradation, the enzyme component system (ECS) of the invention is used with a small amount of added polymerization catalyst.

One component of the enzyme component system (ECS) of the invention used is an enzyme, preferably lipase from *Aspergillus spec.*, at a concentration from 0.05 to 50 mg per liter of wastewater and preferably from 0.05 to 10 mg of enzyme per liter of wastewater (corresponding to about 250 to 50,000 IU per liter of wastewater) (1 IU hydrolyzes 1 μ equivalent of triglyceride fatty acid in 1 hour at pH 7.7 and 37 °C).

The treatment of the grinder wastewater with the enzyme component system of the invention is preferably carried out in the presence of oxygen or air at atmospheric pressure or slight positive oxygen pressure and at a pH from 2 to 11 and preferably from 3 to 6, at a temperature from 20 to 95 °C and preferably from 40 to 95 °C.

Another component is the oxidant, preferably H_2O_2 , which is used at a concentration from 0.05 to 200 mg per liter of wastewater (100% basis) and preferably from 0.05 to 50 mg per liter of wastewater.

Another component consists of one or more fatty acids, preferably C₆ to C₂₆ and particularly C₆ to C₁₆, fatty acids, and more particularly tetradecanoic or dodecanoic acid at a concentration from 0.05 to 200 mg per liter, and preferably at a concentration from 0.05 to 10 mg per liter, of wastewater.

Another component is a ketone, preferably, for example, benzophenone at a concentration from 0.05 to 200 mg per liter of wastewater and preferably at a concentration from 0.05 to 50 mg per liter of wastewater.

Moreover, to increase the efficiency of the process and to use less precipitant (mostly sodium aluminate/aluminum sulfate) which represents the main cost factor, a polymerization catalyst is used, mostly a phenolic substance or a polycyclic compound with several oxidizable hydroxyl groups, in our case preferably, for example, purpurogallin.

These substances are used at a concentration from 0.005 to 200 mg per liter of wastewater and preferably at a concentration from 0.005 to 50 mg per liter of wastewater.

The invention will be further illustrated by way of the following examples:

EXAMPLE 4

190 mL of grinder wastewater was adjusted to pH 6, its temperature was adjusted to 45 °C in an appropriate jacketed reaction vessel and to it were added the following solutions:

- 1) Enzyme solution: lipase (*Aspergillus spee.*): 1 mg in 0.1 mL of water
- 2) Fatty acid solution: 1 mg of dodecanoic acid in 1 mL of water
- 3) Ketone solution: 1 mg of 2,2',4,4'-tetrahydroxybenzophenone in 1 mL of water
- 4) Polymerization catalyst: 0.1 mg of purpurogallin in 0.1 mL of water.

The reaction was initiated by addition of solution 5) (oxidant: H_2O_2), namely of a solution of 3.3 mg of H_2O_2 (30%) in 0.1 mL of water, and the volume was adjusted to 200 mL with preheated water. The reaction was allowed to proceed for 1 to 4 hours and preferably for 2 hours. The wastewater was then either only filtered or filtered and precipitated with 0.2%/0.2% or 0.5%/0.5% aluminum sulfate solution/sodium aluminate solution, 10 wt % in each case and based on the initial value without treatment. The lignin which in untreated grinder wastewater is usually present in an amount from 600 to 900 mg per liter was determined quantitatively by photometry at 280 nm. The drop in lignin is a measure of COD reduction and of the efficiency of the system.

The results are collected in Table 2.

EXAMPLE 5 (without polymerization catalyst)

190 mL of grinder wastewater was adjusted to pH 6, its temperature was adjusted to 45 °C in an appropriate jacketed reaction vessel and to it were added the following solutions:

- 1) Enzyme solution: lipase (*Aspergillus spec.*): 1 mg in 0.1 mL of water
- 2) Fatty acid solution: 1 mg of dodecanoic acid in 1 mL of water
- 3) Ketone solution: 1 mg of 2,2',4,4'-tetrahydroxybenzophenone in 1 mL of water

The reaction was initiated by addition of solution 4) (oxidant- H_2O_2), namely a solution of 3.3 mg of H_2O_2 (30%) in 0.1 mL of water, and the volume was adjusted to 200 mL with preheated water. The reaction was allowed to proceed for 1 to 4 hours and preferably for 2 hours. The wastewater was then either only filtered or filtered and precipitated with 0.2%/0.2% or 0.5%/0.5% aluminum sulfate solution/sodium aluminate solution, 10 wt % in each case based on the initial value without treatment. The lignin which in untreated grinder wastewater is usually present in an amount from 600 to 900 mg per liter was determined quantitatively by photometry at 280 nm. The drop in lignin is a measure of COD reduction and of the efficiency of the system.

The results are collected in Table 2.

TABLE 2

** Polymerization catalyst

III) Use in the Preparation of Lignin Solutions or Gels, Corresponding Binders/Adhesives and of Wood-Based Composites

In this application, too, the polymerization of the lignin or lignin-containing materials is desired and not lignin degradation. Hence, the enzyme component system (ECS) of the invention is used with a small amount of added polymerization catalyst.

Because it was found that the polymerization of lignin, for example in groundwood pulp wastewater (grinder wastewater) is also a good system for evaluating general polymerization properties for the use of the enzyme component system (ECS) in the preparation of lignin solutions or gels, of the corresponding binders/adhesives and of wood-based composites, tests were carried out with the same experimental formulations as for the wastewater.

In this regard, it is known from the cited patents WO 94/01488, WO 93/25622, WO 93/23477 and DE 3 037 992 C2 that, for example, in the production of particle board the binder made by polymerization and dissolution of lignin is applied by spraying it onto the wood fiber material in an amount of about 40 to 100 g per kg of said material which is then subjected to pressing at a pressure of about 20-40 kg / cm² for about 2-4 min and at a temperature rising from about 35 to 190 °C within about 20 seconds. The pressures and temperatures used for pressing can, of course, also be substantially lower, and subsequent curing of the binder/wood fiber mixture by continuing enzyme-catalyzed reactions may be desired.

To evaluate the polymerization properties of ECS for this application, we used as the model system, as stated hereinabove, the above-described system for removing lignin from grinder wastewater.

As one component of the enzyme component system (ECS) of the invention was used an enzyme, preferably lipase from *Humicola lanuginosa*, at a concentration from 0.05 to 50 mg per liter of wastewater and preferably from 0.05 to 10 mg of enzyme per liter of wastewater (corresponding to about 250 to 50,000 IU per liter of wastewater) (1 IU hydrolyzes 1 μ equivalent of triglyceride fatty acid in 1 hour at pH 7.7 and 37 °C).

The treatment of the grinder wastewater with the enzyme component system of the invention is preferably carried out in the presence of oxygen or air at atmospheric pressure or slight positive oxygen pressure and at a pH from 2 to 11 and preferably from 3 to 6, at a temperature from 20 to 95 °C and, preferably from 40 to 95 °C.

Another component is the oxidant, preferably H₂O₂ which is used at a concentration from 0.05 to 200 mg per liter of wastewater (100% basis) and preferably from 0.05 to 50 mg/liter of wastewater.

Another component consists of one or more fatty acids, preferably a C₆ to C₂₆, particularly C₆ to C₁₆ fatty acid and more particularly tetradecanoic or dodecanoic acid at a concentration from 0.05 to 200 mg/liter of wastewater and preferably at a concentration from 0.05 to 50 mg/liter of wastewater.

Another component is a ketone, preferably, for example, benzophenone at a concentration from 0.05 to 200 mg/liter of wastewater and preferably at a concentration from 0.05 to 50 mg/liter of wastewater.

Moreover, as already stated in the foregoing, to increase the efficiency of the process a polymerization catalyst is used, mostly a phenolic substance or a polycyclic compound with several oxidizable hydroxyl groups, in our case preferably, for example, purpurogallin.

These substances are used at a concentration from 0.005 to 200 mg per liter of wastewater and preferably at a concentration from 0.005 to 50 mg per liter of wastewater.

EXAMPLE 6

190 mL of grinder wastewater was adjusted to pH 8.5, preheated to a temperature of 45 °C in an appropriate jacketed reaction vessel and to it were added the following solutions:

- 1) Enzyme solution: lipase (*Humicola lanuginosa*): 1 mg in 0.1 mL of water
- 2) Fatty acid solution: 1 mg of dodecanoic acid in 1 mL of water
- 3) Ketone solution: 1 mg of 2,2',4,4'-tetrahydroxybenzophenone in 1 mL of water
- 4) Polymerization catalyst: 0.1 mg of purpurogallin in 0.1 mL of water.

The reaction was initiated by addition of solution 5) (oxidant: H_2O_2), namely a solution of 3.3 mg of H_2O_2 (30%) in 0.1 mL of water, and the volume was adjusted to 200 mL with preheated water. The reaction was allowed to proceed for 1 to 4 hours and preferably for 2 hours. The wastewater was then either only filtered or filtered and precipitated with 0.2%/0.2% or 0.5%/0.5% aluminum sulfate solution/sodium aluminate solution, 10 wt % in each case, based on the initial value without treatment. The lignin which in untreated grinder wastewater is usually present in an amount from 600 to 900 mg per liter was determined quantitatively by photometry at 280 nm. The drop in lignin is a measure of COD reduction and of the efficiency of the system. The results are collected in Table 2.

IV) Use as Enzymatic Deinking System

In this application, no lignin degradation is desired but, rather, a swelling effect on the lignin-containing fibers so as to bring about the detachment of adhering printing ink particles, an effect similar to that of sodium hydroxide solution in conventional chemical deinking.

To the enzyme component system (ECS) are added besides the usual components such as lipase, oxidant, fatty acid and ketone, also a number of phenolic substances which serve as polymerization catalysts in wastewater treatment and in lignin polymerization/modification applications. Here, we found, surprisingly, that these substances cause a shift in the pH for the optimum enzyme activity thus improving performance.

Also surprisingly, we found that the addition of a reducing agent, preferably dithionite or bisulfite increases the efficiency of ink detachment.

One component of the enzyme component system (ECS) of the invention used is an enzyme, preferably lipase from *Humicola lanuginosa*, at a concentration from 5 to 500 mg per kg of air-dried waste paper, and preferably from 5 to 100 mg of enzyme per kg of waste paper (corresponding to about 25,000 to 500,000 IU per kg of waste paper) (1 IU hydrolyzes 1 μ equivalent of triglyceride fatty acid in 1 hour at PH 7.7 and 37 °C). The treatment of the waste paper with the enzyme component system of the invention for the purpose of removing printing ink particles is preferably carried out in the presence of oxygen or air at atmospheric pressure or slightly positive oxygen

pressure (maximum 2 bar) and at a pH from 7 to 11 and preferably 7 to 9, at a temperature from 20 to 95 °C and preferably from 40 to 95 °C.

Another component is the oxidant, preferably H_2O_2 , which is used at a concentration from 5 to 5000 mg per kg of waste paper (100% basis) and preferably from 5 to 1000 mg per kg of waste paper.

Another component consists of one or more fatty acids, preferably C_6 to C_{26} , and particularly C_6 to C_{16} , fatty acids, and more particularly tetradecanoic or dodecanoic acid at a concentration from 5 to 2000 mg per kg of waste paper and preferably at a concentration from 5 to 500 mg per kg of waste paper.

Another component is a ketone, preferably, for example, benzophenone at a concentration from 5 to 2000 mg per kg of waste paper and preferably at a concentration from 5 to 500 mg per kg of waste paper.

Moreover, to increase the efficiency of the process, the abovesaid compounds are used, for example phenolic substances or polycyclic compounds with several oxidizable hydroxyl groups and preferably, for example, bisphenol A. These substances are employed at a concentration from 1 to 2000 mg per kg of waste paper and preferably at a concentration from 1 to 500 mg per kg of waste paper.

In addition, a reducing agent is used, preferably Na-dithionate or Na-bisulfite, at a concentration from 0.1 to 1000 mg per kg of waste paper and preferably at a concentration from 0.1 to 200 mg per kg of waste paper.

To collect the printing ink particles, commercial detergents are used as collectors, preferably Incopur brands, for example Incopur RSGA, at a concentration from 1 to 5000 mg per kg of waste paper, and preferably from 1 to 1000 mg per kg of waste paper.

To enhance the detaching effect on many waste paper compositions, additional enzymes can be added, for example cellulases and/or hemicellulases (for example, xylanase and/or mannase etc.) and/or pectinases and/or oxidoreductases.

The invention will be further illustrated by way of the following examples:

EXAMPLE 7

About 10 kg of water (preheated to about 45 °C) was added to the pulper of a Lamort laboratory deinking apparatus, and the pH was adjusted with sodium hydroxide solution (and/or sulfuric acid) so that after the addition of 1.5 kg of air-dried waste paper (50% newspapers, 50% magazines) which had been cut into about 2 x 3 cm pieces and after the addition of the other system constituents the pH was 8.0 to 8.5.

These system constituents were (per kg of air-dried waste paper):

- a) 500,000 IU of lipase from *Humicola lanuginosa* per 100 mL of tap water
- b) 0.1 g of dodecanoic acid per 100 mL of tap water
- c) 0.1 g of benzophenone per 100 mL of tap water
- d) 0.1 g of bisphenol A per 20 mL of 0.1 molar NaOH
- e) 0.02 g of Na bisulfite per 10 mL of tap water
- f) 0.5 g of Incopur RSGA per 100 mL of tap water
- g) 1 g of H_2O_2 (30%) per 100 mL of tap water (added at the end).

The pulper was started after the addition of system constituents a) to g) and during the addition of the waste paper. The total quantity of water was then adjusted to 15 kg with approximately 45 °C tap water. The pulping process was allowed to proceed for 10

minutes. For further reaction, the fiber suspension was transferred to a heated vessel where it was allowed to incubate at about 40-45 °C for 15 to 45 minutes. In a Voith flotation cell, 100 g of pulp, absolutely dry basis, (after this incubation) was adjusted to a total volume of about 20 L with tap water (45 °C) and subjected to flotation for 10 to 20 minutes. The accepted stock was discharged, test sheets were prepared from it in a commercial sheet former, dried and the ISO brightness were determined. Table 3 shows the results.

(ISO = International Standardization Organization)

EXAMPLE 8

The method was the same as in Example 7, but instead of the enzyme component system (ECS) of the invention only water was used. Table 3 shows the results.

EXAMPLE 9

About 1 kg of water (preheated to about 45 °C) was added to a dough mixer, and the pH was adjusted with sodium hydroxide solution (and/or sulfuric acid) so that after the addition of 150 g of air-dried waste paper (50% newspapers, 50% magazines) which had been cut into about 2 x 3 cm pieces and after the addition of the other system constituents the pH was 8.0 to 8.5.

These system constituents were (per 100 g of air-dried waste paper):

a) 5000 IU of amidase from *Pseudomonas aeruginosa* (Sigma A 6691) per 100 mL of tap water

(1 IU = conversion of 1 μ mole of acetamide and hydroxylamine to acetohydroxamic acid and NH_3 per minute at pH 7.2 and 37 °C)

- b) 0.01 g of dodecanoic acid per 100 mL of tap water
- c) 0.01 g of benzophenone per 100 mL of tap water
- d) 0.01 g of bisphenol A per 20 mL of 0.1 molar NaOH
- e) 0.002 g of Na bisulfite per 10 mL of tap water
- f) 0.05 g of Incopur RSGA per 100 mL of tap water
- g) 0.1 g of H_2O_2 (30%) per 100 mL of tap water (added at the end).

The dough mixer was started after the addition of system constituents a) to g) and during the addition of the waste paper. The total quantity of water was then adjusted to 1.5 kg with tap water (45 °C). The pulping process was allowed to proceed for 10 minutes.

For further reaction, the fiber suspension was transferred to a heated vessel where it was allowed to incubate at about 40-45 °C for 15 to 45 minutes.

In a Voith flotation cell, 100 g of pulp, absolutely dry basis, (after this incubation) was adjusted to a total volume of about 20 L with tap water (45 °C) and subjected to flotation for 10 to 20 minutes. The accepted stock was discharged, test sheets were prepared from it in a commercial sheet former, dried and the ISO brightness and were determined. Table 3 shows the results.

EXAMPLE 10 (Chemical System)

About 10 kg of water (preheated to about 45 °C) was added to the pulper of a Lamort laboratory deinking apparatus, and 1.5 kg of air-dried waste paper (50% newspapers, 50% magazines), cut into approximately 2 x 3 cm pieces, was added after the addition of the following chemicals (based on air-dried pulp):

- 1) 0.8 wt % of soap (DR 3, Henkel)
- 2) 3.5% of water glass
- 3) 2% of sodium hydroxide (100%)
- 4) 1 % H₂O₂ (100%)

The pulper was started during waste paper addition. The total quantity of water was then adjusted to 15 kg with about 45 °C tap water. The pulping process was allowed to proceed for 10 minutes.

For further reaction, the fiber suspension was transferred to a heated vessel where it was allowed to incubate at about 40-45 °C for 15 to 45 minutes,

In a Voith flotation cell, 100 g of pulp, absolutely dry basis, (after this incubation) was adjusted to a total volume of about 20 L with tap water (45 °C) and subjected to flotation for 10 to 20 minutes. The accepted stock was discharged, test sheets were prepared from it in a commercial sheet former, dried and the ISO brightness were determined. Table 3 shows the results.

TABLE 3

System	ISO Brightness, %
Water only	51
Chemical system	61
ECS/lipase	58.5
ECS/amidase	57
Comparative system: laccase (800,000 IU/kg of waste paper + bisphenol A + Na bisulfite (0.1 or 0.02 g/kg of waste paper), for other con- ditions see WO 91/14820; WO 92/20857	55.5

V) Use as Oxidation System in Organic Synthesis

From the multiplicity of possible uses of the enzyme component system (ECS) of the invention, such as in hydroxylation reactions, oxidation of unsaturated aliphatics, Baeyer-Villiger oxidations, oxidation of heterocycles, carbon-carbon dehydrogenations

and other oxidation reactions, by way of the following examples, the oxidation of alcohols to aldehydes and of aromatic methyl groups to aldehydes are described.

It is known from the literature that these reactions can be carried out with the oxidoreductase laccase and a mediator such as ABTS (2,2'-azino-bis(3-ethylbenzothiazolin-6-sulfonic acid), see T. Rosenau et al., Synthetic Communications 26 (2), 315-320 (1996) and A Potthast et al., J. Org. Chem. (60), pp. 4320-4321 (1995). The main advantage of the process of the invention over these processes is its lower cost and better performance, particularly based on the cost.

One of the components of the enzyme component system (ECS) of the invention is an enzyme, preferably lipase from, for example, *Humicola lanuginosa*, used at a concentration of 0.05 to 5 mg per 10 mmoles of substrate, preferably from 0.05 to 3 mg per 10 mmoles of substrate (which corresponds to about 250 to 15,000 IU) (1 IU hydrolyzes 1 μ equivalent of a triglyceride fatty acid in 1 hour at pH 7.7 and 37 °C). Preferably, the oxidation reaction with the enzyme component system of the invention is carried out in the presence of oxygen or air at atmospheric pressure or a slightly positive O₂ pressure and at pH from 2 to 11, preferably at pH 3-9, at a temperature of 20 to 95 °C, preferably 40-95 °C and a 5 to 100 mmolar, preferably 5 to 50 mmolar, substrate concentration.

Another component is the oxidant, preferably H₂O₂ (100%), which is used at a concentration from 0.05 to 100 mg per 10 mmoles of substrate and preferably from 0.05 to 30 mg per 10 mmoles of substrate.

Another component consists of one or more fatty acids, preferably C₆ to C₂₆, and particularly C₆ to C₁₆ fatty acids, and more particularly tetradecanoic or dodecanoic acid at a concentration from 0.05 to 100 mg per 10 mmoles of substrate and preferably at a concentration from 0.05 to 30 mg per 10 mmoles of substrate.

Another component is a ketone, preferably, for example, benzophenone at a concentration from 0.05 to 100 mg per 10 mmoles of substrate and preferably at a concentration from 0.05 to 30 mg per 10 mmoles of substrate.

The invention will be further illustrated by way of the following examples:

EXAMPLE 11 (Oxidation of Benzyl Alcohols to Aldehydes)

The following components were added to 50 mL of 0.1 molar pH 4.5 acetate buffer in a 250-mL reaction flask:

- 1) p-methoxybenzyl alcohol in 30 mL of tetrahydrofuran [THF] (20-molar in total volume)
- 2) 2 mg of lipase from *Humicola lanuginosa*
- 3) 5 mg of dodecanoic acid
- 4) 25 mg of benzophenone

The reaction was started by addition of 12.5 mg of H₂O₂ (30%) and was allowed to proceed for 12 to 24 hours. Then, 0.5 mL of the reaction solution was removed, extracted with CH₂Cl₂, and the p-methoxybenzaldehyde content was determined by GC or GC-MS. The results are shown in Table 4.

EXAMPLE 12 (Oxidation of Aromatic Methyl Groups to Aldehydes)

The following components were added to 50 mL of 0.1 molar pH 4.5 acetate buffer in a 250-mL reaction flask:

- 1) toluene in 30 mL of THF (20-molar in total volume)
- 2) 2 mg of lipase from *Humicola lanuginosa*
- 3) 5 mg of dodecanoic acid
- 4) 25 mg of benzophenone

The reaction was started by addition of 12.5 mg of H_2O_2 (30%) and was allowed to proceed for 12 to 24 hours. Then, 0.5 mL of the reaction solution was removed, extracted with CH_2Cl_2 , and the benzaldehyde content was determined by GC or GC-MS. The results are shown in Table 4.

TABLE 4

Substrate	Oxidized Substrate	Conversion, %
P-Methoxybenzyl alcohol (lipase)	p-methoxybenzaldehyde	98
p-Methoxybenzyl alcohol (ABTS/laccase)	p-methoxybenzaldehyde	90
Toluene (lipase)	benzaldehyde	98
Toluene (ABTS/laccase)	benzaldehyde	92

VI) Use in Coal Liquefaction

Recently, the use of white rotting fungi was studied in the liquefaction of lignite and anthracite, and the general feasibility thereof was confirmed. Patent applications WO 94/29510 and WO 96/18770 have also disclosed the general feasibility of using fungus-free systems based on oxido- reductases and special mediators.

Surprisingly, we were able to confirm for the enzyme component system (ECS) of the invention that it, too, can be used for "liquefying" the lignin-like tridimensional network of polycyclic aromatic ring systems of lignite and anthracite.

One of the components of the enzyme component system (ECS) of the invention is an enzyme, preferably lipase from, for example, *Humicola lanuginosa*, used at a concentration of 0.05 to 20 mg per gram of ground lignite, absolutely dry basis, preferably from 0.05 to 10 mg per gram of coal (corresponding to about 250 to 50,000 IU) (1 IU hydrolyzes 1 μ equivalent of a triglyceride fatty acid in 1 hour at pH 7.7 and 37 °C).

Preferably, the coal treatment with the enzyme component system of the invention is carried out in the presence of oxygen or air at atmospheric pressure or at a slightly positive O₂ pressure and at pH from 2 to 11, preferably at pH 3-9, at a temperature of 20 to 95 °C, preferably 40-95 °C, and a coal consistency of 0.5 to 40%.

An unusual and surprising finding concerning the use of enzymes is that when the enzyme component system of the invention is employed, the consistency of the material can be increased and the performance is markedly improved. For economic reasons, the process according to the invention is carried out at a coal consistency from 4 to 35% and particularly from 4 to 15%.

Another component is the oxidant, preferably H₂O₂, which is used at a concentration from 0.05 to 100 mg per gram of coal (100% basis) and preferably from 0.05 to 50 mg per gram of coal.

Another component consists of one or more fatty acids, preferably C₆ to C₂₆, particularly C₆ to C₁₆ fatty acids, and more particularly tetradecanoic or dodecanoic acid at a concentration from 0.05 to 100 mg per gram of coal and preferably at a concentration from 0.05 to 50 mg per gram of coal.

Another component is a ketone, preferably, for example, benzophenone at a concentration from 0.05 to 100 mg per gram of coal and preferably at a concentration from 0.05 to 50 mg per gram of coal.

The invention will be further illustrated by way of the following example:

EXAMPLE 13

Enzymatic Coal Liquefaction

5 g of lignite or anthracite, absolutely dry basis, (particle size about 200 to 500,μ) was added to solutions prepared as follows:

A) To 20 mL of tap water were added 5 mg of tetradecanoic acid, 25 mg of benzophenone and 12.5 mg of H₂O₂ (30%) per gram of coal with agitation. The pH was adjusted with sulfuric acid and/or sodium hydroxide solution so that, after addition of the coal and the enzyme, the pH was 8.

B) To 5 mL of tap water was added 10 mg of lipase from *Humicola lanuginosa* (about 50,000 IU).

Solutions A and B were combined and diluted to 45 mL. After addition of the coal, the material was mixed for 2 minutes. The material was then transferred to a reaction vessel preheated to 45 °C and allowed to incubate 1-4 hours.

The resulting coal of modified consistency was then removed from the reaction flask.

VII) Use as Bleaching Agent in Detergents

In using the enzyme component System (ECS) of the invention as a bleaching agent in detergents, one of the components is an enzyme, preferably lipase from, for example, *Humicola lanuginosa*, used at a concentration from 0.05 to 20 mg per 100 mL of washing solution and preferably from 0.05 to 10 mg of enzyme per 100 mL of washing solution (which corresponds to about 250 to 100,000 IU per 100 mL of washing solution) (1 IU hydrolyzes 1 μ equivalent of a triglyceride fatty acid in 1 hour at PH 7.7 and 37 °C).

Preferably, the bleaching with the enzyme component system of the invention is carried out in the presence of oxygen or air at atmospheric pressure or at a slightly positive O_2 pressure and at pH from 2 to 12, preferably at pH 3-10, at a temperature of 20 to 95 °C and preferably 30-95 °C.

Another component is the oxidant, preferably H_2O_2 , which is used at a concentration from 0.05 to 50 mg per 100 mL of washing solution (100% basis) and preferably from 0.05 to 20 mg per 100 mL of washing solution.

Another component consists of one or more fatty acids, preferably a C_6 to C_{26} , particularly a C_6 to C_{16} fatty acid and more particularly tetradecanoic or dodecanoic acid, at a concentration from 0.05 to 50 mg per 100 mL of washing solution and preferably at a concentration from 0.05 to 20 mg per 100 mL of washing solution.

Another component is a ketone, preferably, for example, benzophenone at a concentration from 0.05 to 50 mg per 100 mL of washing solution and preferably at a concentration from 0.05 to 20 mg per 100 mL of washing solution.

Other Components

The bleaching system can also contain phenolic and/or nonphenolic compounds with one or more benzene rings. The following oxidants besides those mentioned hereinabove are particularly preferred: air, oxygen, H_2O_2 , organic peroxides, sodium perborate and/or sodium percarbonate. Oxygen can also be generated in situ by H_2O_2 + catalase or the like, or H_2O_2 can be generated in situ by GOD + glucose or similar systems.

Also preferred are multicomponent bleaching systems containing cation-generating metals salts. The cations Fe^{2+} , Fe^{3+} , Mn^{2+} , Mn^{3+} , Mn^{4+} , Cu^{1+} , Cu^{2+} , Ti^{3+} , Ce^{4+} , Mg^{2+} and Al^{3+} are preferably used.

The bleaching system can also contain polysaccharides and/or proteins. Suitable polysaccharides are, glucans, mannans, dextrans, levans, pectins, alginates, vegetable gums and/or polysaccharides formed by fungi or produced in mixed cultures with yeasts. Suitable proteins are gelatins and albumins, among others. Also suitable are monosaccharides, oligosaccharides, amino acids, PEG, polyethylene oxides, polyethyleneimines and polydimethylsiloxanes.

Use of the Multicomponent System

The multicomponent system can be used in combination with surface-active detergent constituents or detergent additives, which in themselves are known.

The invention will be further illustrated by way of the following examples:

EXAMPLE 14

Effect of ECS on Tea-Stained Standard Cotton Fabrics

A (5x5 cm) piece of fabric was allowed to incubate in 100 mL of washing solution (in a 300-mL Erlenmeyer flask) at 40 °C for 40 min with reciprocating shaking (120 rpm). Before the beginning of incubation, the washing solution was subjected to a 10-min temperature equilibration period.

The washing solution was prepared with standard tap water (STW) at 14° dH'. The following enzyme doses were used: 2.5 mg of lipase from *Humicola lanuginosa*/100 mL, 2.5 mg of tetradecanoic acid/100 mL, 12.5 mg of benzophenone/100 mL and 6.5 mg of H₂O₂ (30%). After decanting the washing liquor", cold water was added 3x in the form of a strong water jet and then decanted.

The results are shown in Table 5.

EXAMPLE 15

Effect of ECS (Amidase) on Tea-Stained Standard Cotton Fabrics

A (5x5 cm) piece of fabric was allowed to incubate in 100 mL of washing solution (in a 300-mL Erlenmeyer flask) at 40 °C for 40 min with reciprocating shaking (120 rpm). Before the beginning of incubation, the washing solution was subjected to a 10-min temperature equilibration period.

The washing solution was prepared with standard tap water (STW) at 14° dH. The following doses were used: 1000 IU of amidase/ 100 mL (1 IU = conversion of 1 μmole of acetamide and hydroxylamine to acetohydroxamic acid per minute at pH 7.2 and 37 ° C), 2.5 mg of tetradecanoic acid/1 00 mL, 12.5 mg of benzophenone/100 mL and 6.5 mg of H₂O₂ (30%). After decanting the "washing liquor", cold water was added 3 x in the form of a strong water jet and then decanted.

The results are shown in Table 5.

(dH = one degree of German water hardness = 10 mg of CaO/L)

TABLE 5

	pH	Whiteness	Brightness
STW zero value	4.5	2.55	2.3
Heavy-duty detergent	10.1	8.9	6.15
STW + ECS (lipase)	8.5	7.5	7.2
STW + ECS (amidase)	8	6.9	6.3
Liquid detergent + ECS (lipase)	8.5	8.5	8.0
Comparative test: Liquid detergent + laccase + HOBT (conditions as in PCT/EP 96/02658; PCT/EP 94101967	5	6.5	6.0

VIII) Use in the Bleaching/Decolorizing of Textiles

One of the components of the enzyme component system (ECS) of the invention used in the bleaching/decolorizing of textile fabrics is an enzyme, preferably lipase from, for example, *Humicola lanuginosa*, employed at a concentration of 0.05 to 10 mg per gram of denim (corresponding to about 250 to 25,000 IU per gram of denim) (1 IU hydrolyzes 1 μ equivalent of a triglyceride fatty acid in 1 hour at pH 7.7 and 37 °C). Preferably, the bleaching/decolorizing with the enzyme component system of the invention is carried out in the presence of oxygen or air at atmospheric pressure or at a slightly positive O₂ pressure and at pH from 2 to 11, preferably at pH 3-9, at a temperature of 20 to 95 °C, preferably 40-95 °C and a fabric density of 0.5 to 40% [fabric density = ratio of weight of fabric to weight of solution]

An unusual and surprising finding concerning the use of enzymes is that when the enzyme component system of the invention is used, the fabric density can be increased and the performance markedly improved. For economic reasons, the process according to the invention is carried out at a fabric density from 4 to 35% and particularly from 4 to 15%.

Another component is the oxidant, preferably H₂O₂, which is used at a concentration from 0.05 to 20 mg per gram of denim (100% basis) and preferably from 0.05 to 10 mg per gram of denim.

Another component consists of one or more fatty acids, preferably C₆ to C₂₆ and particularly C₆ to C₁₆ fatty acids, and more particularly tetradecanoic or dodecanoic acid at a concentration from 0.05 to 20 mg/g of denim and preferably at a concentration from 0.05 to 10 mg per gram of denim.

Another component is a ketone, preferably, for example, benzophenone, used at a concentration from 0.05 to 20 mg/g of denim and preferably at a concentration from 0.05 to 10 mg/g of denim.

The invention will be further illustrated by way of the following examples:

EXAMPLE 16

Bleaching with ECS + Lipase

1 g of denim fabric was placed in a 200-mL Erlenmeyer flask (fabric density 2%). The pH of the solution (tap water), the volume of which was 50 mL after the addition of all components, was preadjusted to pH 6 with 0.5 N H₂SO₄.

1 mg of lipase from *Humicola lanuginosa*, 0.5 mg of tetradecanoic acid, 1 mg of benzophenone and 2.5 mg of H₂O₂ (30%) were added per gram of denim. The experiment was carried out in a shaking water bath (200 rpm) at 45 °C and a reaction time of 45 minutes. The piece of fabric was washed with tap water and dried in air. The brightness was then determined with an Elrepho instrument. The values obtained are given in Table 6.

EXAMPLE 17

Bleaching with ECS + Amidase

1 g of denim fabric was placed in a 200-mL Erlenmeyer flask (fabric density 2%). The pH of the solution (tap water), the volume of which was 50 mL after the addition of all components, was preadjusted to pH 6 with 0.5 N H₂SO₄.

200 IU of amidase from *Pseudomonas aeruginosa* (Sigma A 6691), 0.5 mg of tetradecanoic acid, 1 mg of benzophenone and 2.5 mg of H₂O₂ (30%) were added per gram of denim. The experiment was carried out in a shaking water bath (200 rpm) at 45 °C and a reaction time of 45 minutes. The piece of fabric was washed with tap water and dried in air. The brightness was then determined with an Elrepho instrument. The values obtained are given in Table 6.

TABLE 6

System	pH	ISO Brightness
Untreated specimen	--	4.5
Laccase + violuric acid (comparative system)	3.5	13.5
ECS system + lipase	6.0	16.9
ECS system + amidase	6.0	14.5
Hypochlorite	4.5	n.d.

Addition of Other Substances to the Enzyme Component System (ECS)

For all applications, the components of the enzymatic oxidation systems with enzyme action-enhancing compounds disclosed in DE 198 21 263.1, DE 198 20 947.9 and PCT/DE 98/01313 can be added to the enzyme component system (ECS) of the invention, such systems containing the following:

a) At least one oxidation catalyst, preferably enzymes such as oxidoreductases of classes 1.1.1. to 1.97 according to the International Enzyme Nomenclature: Committee of the International Union of Biochemistry and Molecular Biology (Enzyme Nomenclature, Academic Press, Inc., 1992, pp. 24-154) among which the following are particularly preferred: cellobiose: oxygen-1-oxidoreductase (cellobiose reductase) **1.1.3.25**, cellobiose: quinone-1-oxidoreductase **1.1.5.1**, bilirubin oxidase **1.3.3.5**, cytochrome oxidase **1.9.3**, oxygenases, lipoxxygenases **1.13**, **1.14**, superoxide dismutase **1.15.11**, ferrioxidase, for example ceruloplasmin **1.16.3.1**, especially preferred being the enzymes of class **1.10** which act on related compounds. They catalyze the oxidation of biphenols and ascorbates. Suitable acceptors are NAD⁺, NADP⁺ (**1.10.1**), cytochrome (**1.10.2**), oxygen (**1.10.3**) or others (**1.10.99**). Among these, particularly preferred as acceptors are the enzymes of class **1.10.3** with oxygen (O₂) as acceptor.

Other particularly preferred enzymes are those of group 1.11 which act on a peroxide as acceptor. Only subclass (1.11.1) contains peroxidases. Especially preferred here are cytochrome C peroxidases (1.11.1.5), catalase (1.11.1.6), peroxidase (1.11.1.7), iodide peroxidase, (1.11.1.8), glutathione peroxidase (1.11.1.9), chloride peroxidase (1.11.1.10), L-ascorbate peroxidase (1.11.1.11), phospholipid hydroperoxide glutathione peroxidase (1.11.1.12), manganese peroxidase (1.11.1.13) and diarylpropane peroxidase (ligninase, lignin peroxidase) (1.11.1.14).

c) At least one mediator selected from the group consisting of hydroxylamines, hydroxylamine derivatives, hydroxamic acids, hydroxamic acid derivatives, aliphatic, cycloaliphatic, heterocyclic or aromatic compounds containing at least one N-hydroxy, oxime, N-oxy or N,N'-dioxy function and/or at least one mediator from the group of amides, such as, for example, hydrazides or 1,2,4-triazolidin-3,5-diones (urazoles) and/or at least one mediator from the group of imides such as, for example, the hydantoin, and/or at least one mediator from the group of oxocarbons. Moreover, it is possible to use at least one **mediation enhancer** selected from the group consisting of carbonyl compounds, aliphatic ethers, phenol ethers or olefins (alkenes), and/or at least one **mediation enhancer** selected from the group consisting of the abovesaid mediators of the NO-, NOH- or HRN-OH type and/or amides such as the hydrazides or urazoles and/or the imides such as the hydantoin and/or the oxocarbons.

It is also possible to use at least one mediation enhancer selected from the group consisting of cation radical-generating substances of the phenothiazine and/or phenoxazine type and/or of the (R = N-N = R) type* (for example, ABTS) or of aryl-substituted alcohols (nonphenols) such as, for example, veratryl alcohol and/or phenol derivatives, such as p-hydroxycinnamic acid, 2,4-dichlorophenol, p-hydroxybenzenesulfonate, vanillin (4-hydroxy-3-methoxybenzaldehyde), p-hydroxybenzoic acid, 5- amino-2-hydroxybenzoic acid (5-aminosalicylic acid) and/or Wurster-type radical cation compounds [see *Angewandte Chemie* 91, pp. 982-997 (1979); *Chem. Unserer Zeit* 12, pp. 89-98 (1978); *Römpf Chemie Lexikon* [*Römpf Chemical Encyclopedia*, 9th edition, 1995) and/or radical anions, for example semiquinones formed by enzymatic oxidation of hydroquinones.

It is essential for improving the performance of the enzyme/mediator systems with the aid of comediators that the mediator/comediator ratio be from 5000 : 1 to 1 : 1, a ratio from 500 : 1 to 1 : 1 being particularly preferred. When several mediators and comediators are used at the same time, the ratio of these mediator or comediator concentrations depends on the particular combinations employed.

* N means nitrogen, R denotes groups.

The pulp was washed over a nylon screen (30 μ m) and extracted for one hour at 60 °C, 2% pulp consistency and using 8% NaOH per gram of wood pulp. The pulp was again washed, after which the kappa number was determined. For results see Table 7.

EXAMPLE 19 (Enzyme: lipase/peroxidase)

Enzymatic Bleaching of Softwood (Sulfate Pulp)

5 g, absolutely dry basis, of wood pulp (O₂-delignified softwood), pulp consistency 30% (about 17 g moist) was added to solutions prepared as follows:

A) To 20 mL of tap water were added 1 mg of tetradecanoic acid, 5 mg of benzophenone, 2.5 mg of H₂O₂ (30%), 37 μmoles of violuric acid + 0.37 μmole of 4-tert.butylurazole with agitation. The pH was adjusted with 0.5 mole/L sulfuric acid solution so that, after addition of the wood pulp and the enzyme, the pH was 7.

B) To 5 mL of tap water was added 5 mg of lipase from *Humicola lanuginosa* (about 25,000 IU) and 0.1 mg of peroxidase (horseradish) per gram of wood pulp. Solutions A and B were combined and diluted to 33 mL. After addition of the wood pulp, the material was mixed in a dough mixer for 2 minutes. The material was then transferred to a reaction vessel preheated to 45 °C and allowed to incubate 1-4 hours at atmospheric pressure.

The pulp was washed over a nylon screen (30 μm) and extracted for one hour at 60 °C, 2% pulp consistency and using 8% NaOH per gram of wood pulp. The pulp was again washed, after which the kappa number was determined. For results see Table 7.

TABLE 7

SYSTEM	% DELIG. (lipase/lacc.)	% DELIG. (lipase/peroxid.)
ECS + lipase system (+ laccase + violuric acid) +*	44	---
ECS + lipase system (+ peroxidase + violuric acid) +*	---	38
Laccase (+ violuric acid) +*	27	---
Peroxidase (+ violuric acid) +*	---	28

* **Mediation enhancer** 4-tert.butylurazole

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APPENDIX I

System Component 2 of the Enzyme Component System (ECS) of the Invention

The fatty acids used in the process of the invention as a source of peracid are, for example:

1) Saturated fatty acids

Butanoic acid	(butyric acid)
Pentanoic acid	(valeric acid)
Hexanoic acid	(caproic acid)
Heptanoic acid	(enanthic acid)
Octanoic acid	(caprylic acid)
Nonanoic acid	(pelargonic acid)
Decanoic acid	(capric acid)
Undecanoic acid	
Dodecanoic acid	(lauric acid)
Tridecanoic acid	
Tetradecanoic acid	(myristic acid)
Pentadecanoic acid	
Hexadecanoic acid	(palmitic acid)
Heptadecanoic acid	
Octadecanoic acid	(stearic acid)
Nonadecanoic acid	
Eicosanoic acid	(arachic acid)
Heneicosanoic acid	
Docosanoic acid	(behenic acid)
Tricosanoic acid	
Tetracosanoic acid	(lignoceric acid)
Pentacosanoic acid	
Hexacosanoic acid	(cerotic acid)
Octacosanoic acid	
Triacosanoic acid	(melissic acid)

2) Unsaturated fatty acids

10-Undecenoic acid	
9-cis-Dodecenoic acid	(lauroleic acid)
9-cis-Tetradecenoic acid	(myristoleic acid)
9-cis-Hexadecenoic acid	(paimitoleic acid)
6-cis-Octadecenoic acid	(petroselic acid)
6-trans-Octadecenoic acid	(petroselaidic acid)
9-cis-Octadecenoic acid	(oleic acid)
9-trans-Octadecenoic acid	(elaidic acid)
9-cis, 12 cis-Octadecadienoic acid	(linoleic acid)
9-trans, 1 2-trans-Octadecadienoic acid	(linolaidic acid)
9-cis, 12-cis, 15-cis-Octadecatrienoic acid	(linolenic acid)
9-trans, 11 -trans, 1 3-trans- Octadecatrienoic acid	(α -eleostearic acid)
9-cis, 11 -trans, 1 3-trans-Octadecatrienoic acid	(β -eleostearic acid)

9-cis-Icosenic acid	(gadoleic acid)
Icosa-5,8,11,14-tetraenoic acid	(arachidic acid)
13-cis-Docosenoic acid	(erucic acid)
13-trans-Docosenoic acid	(brassicic acid)
4,8,12,15,19-Docosapentaenoic acid	(clupanodonic acid)

3) Polyunsaturated fatty acids

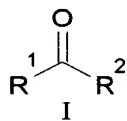
9,12-Octadecadienoic acid	(linoleic acid)
9,12,15-Octadecatrienoic acid	(linolenic acid)
5,9,12-Octadecatrienoic acid	
9,11,13-Octadecatrienoic acid	(eleostearic acid)
9,11,13,15-Octadecatetraenoic acid	(parinaric acid)
5,11,14-Icosatrienoic acid	
5,8,11,14-Icosatetraenoic acid	(arachidic acid)
4,8,12,15,18-Icosapentaenoic acid	
4,8,12,15,19-Docosapentaenoic acid	(clupanodonic acid)
4,8,12,15,18,21-Tetracosahexaenoic acid	(nisinic acid)

Particularly preferred are tetradecanoic acid (myristic acid) and dodecanoic acid (lauric acid).

APPENDIX II

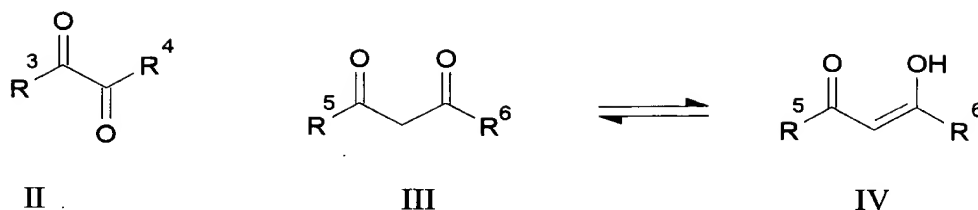
System Component 4 (Ketones) of the Enzyme Component System (ECS) of the Invention

Particularly preferred are carbonyl compounds of general formula I:



The R^1 and R^2 groups can be equal or different and denote aliphatic or aromatic groups. Moreover, the R^1 and R^2 groups can form a ring containing besides carbon also heteroatoms such as nitrogen, oxygen and sulfur.

Particularly preferred are 1,2-diketones (formula II), 1,3-diketones (formula III), polyketones (polyketides) and the tautomeric enols (formula IV):



wherein the R^3 to R^6 groups, once again, can be equal or different and denote aliphatic or aromatic groups. Moreover, groups R^3 and R^4 and groups R^5 and R^6 , together, can form a ring containing besides carbon also heteroatoms such as nitrogen, oxygen or sulfur. The possibility of tautomerization or formation of a resonance hybrid is particularly important in this case.

Besides general carbonyl compounds, particularly preferred are ketones, such as, in general hydroxyketones, α,β -unsaturated ketones, oxycarboxylic acids, quinones and halogenated ketones.

Particularly preferred among these are the following:

Acetone, methyl ethyl ketone, diethyl ketone, methyl n-butyl ketone, methyl isobutyl ketone, cyclohexanone, cyclopentanone, 2-methylcyclohexanone, 3-methylcyclohexanone, 4-methylcyclohexanone, dihydroxyacetone, diacetyl monohydrazone, diacetyl dihydrazone, acetophenone, p-hydroxyacetophenone, 1-phenyl-3-butanone, 3-pentanone, 4-heptanone, 2-nonanone, cycloheptanone, cyclooctanone, cyclodecanone, cyclododecanone, dimethyl ketone, ethyl propyl ketone, methyl amyl ketone, acetylacetone, pinacolone, methyl isopropyl ketone, methyl isoamyl ketone, ethyl amyl ketone diisopropyl ketone, diisobutyl ketone, methyl vinyl ketone, methyl isopropenyl ketone, mesityl oxide, isophorone, hydroxyacetone, methoxyacetone, 2,3-pentanedione, 2,3-hexanedione, phenylacetone, propiophenone, benzophenone, benzoin, benzil, 4,4'-dimethoxybenzil, 4'-methoxyacetophenone, 3'-methoxyacetophenone, O-ethylbenzoin, (2-methoxyphenyl)acetone, (4-methoxyphenyl)acetone, methoxy-2-propanone, glyoxylic acid, benzyl glyoxylate, benzylacetone, methyl benzyl ketone, methylcyclohexyl ketone, 2-decanone, dicyclohexyl ketone, 3,3-dimethyl-2-butanone, methyl isobutyl ketone, methyl isopropyl ketone, 2-methyl-3-heptanone, 5-methyl-3-heptanone, 6-methyl-5-hepten-2-one, 5-methyl-2-hexanone, 3-nonanone, 5-nonanone, 2-octanone, 3-octanone, 2-undecanone, 1,3-dichloroacetone, 1-hydroxy-2-butanone, 3-hydroxy-2-butanone, 4-hydroxy-4-methyl-2-pentanone, 2-(1S)-adamanantone, anthrone, bicyclo(3.2.0)hept-2-en-6-one, cis-bicyclo(3.3.0)octan-3,7-dione, (1S)-(-)-camphor, p-chloranil, cyclobutanone, 1,3-cyclohexanedione, 1,4-cyclohexanedione monoethylene ketal, dibenzosuberone, ethyl 4-oxocyclohexanecarboxylate, 9-fluorenone, 1,3-indandione, methylcyclohexanone, phenylcyclohexanone, 4-propylcyclohexanone, 1,2,3,4-tetrahydro-1-naphthalenone, 1,2,3,4-tetrahydro-2-naphthalenone, 3,3,5-trimethylcyclohexanone, 3-acetoxy-2-cyclohexen-1-one, benzylideneacetone, (R)-(-)-carvone,

(S)-(-)-carvone, curcumin, 2-cyclohexen-1-one, 2,3-diphenyl-2-cyclopropen-1-one, 2-hydroxy-3-methyl-2-cyclopentene-1-one, isophorone, α -ionone, β -ionone, 3-methoxy-2-cyclohexen-1-one, 3-methyl-2-cyclopenten-1-one, 3-methyl-3-penten-2-one, (R)-(+)-pulegone, tetraphenyl-2,4-cyclopentadien-1-one, 2,6,6-trimethyl-2-cyclohexen-1,4-dione, 2-acetylbenzoic acid, 1-acetylnaphthalene, 2-acetylnaphthalene, 3'-aminoacetophenone, 4'-aminoacetophenone, 4'-cyclohexylacetophenone, 3',4'-diacetoxyacetophenone, diacetylbenzene, 2',4'-dihydroxyacetophenone, 2',5'-dihydroxyacetophenone, 2',6'-dihydroxyacetophenone, 3,4-dimethoxyacetophenone, 2'-hydroxyacetophenone, 4'-hydroxyacetophenone, 3'-methoxyacetophenone, 4'-methoxyacetophenone, 2'-methylacetophenone, 4'-methylacetophenone, 2'-nitroacetophenone, 3'-nitroacetophenone, 4'-phenylacetophenone, 3,4',5'-trimethoxyacetophenone, 4'-aminopropiophenone, benzoylacetone, benzoylpropionic acid, benzylideneacetophenone, cyclohexyl phenyl ketone, desoxybenzoin, 4',4'-dimethoxybenzil, 1,3-diphenyl-1,3-propanedione, ethylbenzoyl acetate, ethyl phenylglyoxylate, 4'-hydroxypropiophenone, 1,3-indandione, 1-indanone, isopropyl phenyl ketone, 6-methoxy-1,2,3,4-tetrahydronaphthalen-1-one, methylphenyl glyoxylate, phenylglyoxylonitrile, 1-phenyl-1,2-propanedione 2-oxime, valerophenone, 2-acetyl- γ -butyrolactone, 2-acetylpyrrole, 1-benzylpiperidin-4-one, dehydroacetic acid, 3,4-dihydro-4,4-dimethyl-2H-pyran-2-one, 1,4-dihydro-4-pyridinone, N-ethoxycarbonyl-4-piperidinone, 2-methyl furyl ketone, 5-hydroxy-2-hydroxymethyl-4H-pyran-4-one, 3-hydroxy-2-methyl-4-pyranone, 3-indolyl methyl ketone, isatin, 1-methyl-4-piperidinone, methyl 2-pyridyl ketone, methyl 3-pyridyl ketone, methyl 4-pyridyl ketone, methyl 2-thienyl ketone, phenyl 2-pyridyl ketone, phenyl 4-pyridyl ketone, tetrahydrofuran-2,4-dione, tetrahydro-4H-pyran-4-one, 2,2,6,6-tetramethyl-4-piperidone, xanthone, acenaphthene quinone, pyruvic acid, (1 R)-(-)-camphor quinone, (1S)-(+)-camphor quinone, 3,5-ditert.butyl-o-benzoquinone, 1,2-dihydroxy-3,4-cyclobutendione, ethyl (2-amino-4-thiazolyl)glyoxylate, ethyl pyruvate, 2,3-hexanedione, 3,4-hexanedione, 3-methyl-2-oxobutyric acid, 3-methyl-2-oxovaleric acid, 4-methyl-2-oxovaleric acid, 2-oxobutyric acid, 2,3-pentandione, 9,10-phenanthrene quinone, acetoacetanilide, 2-acetyl- γ -butyrolactone, 2-acetylcyclopentanone, allyl acetoacetate, benzoylacetone, tert.butyl acetoacetate, 1,3-cyclopentanedione, diethyl 3-oxoglutarate, dimethyl acetylsuccinate, dimethyl 3-oxoglutarate, 1,3-diphenyl-1,3-propanedione, ethyl acetoacetate, ethyl benzoylacetate, ethyl butyrylacetate, ethyl 2-oxocyclohexanecarboxylate, ethyl 2-phenylacetoacetate, methyl acetoacetate, 2-methyl-1,3-cyclohexanedione, 2-methyl-1,3-cyclopentanedione, methyl isobutyrylacetate, methyl 3-oxopentanoate, methyl pivaloylacetate, 3-oxoglutaric acid, tetrahydrofuran-2,4-dione, 2,2,6,6-tetramethyl-3,5-heptanedione, 3-benzoylpropionic acid, 1,4-cyclohexanedione, dimethyl acetylsuccinate, ethyl levulinate, 2-aminoanthraquinone, anthraquinone, p-benzoquinone, 1,4-dihydroxyanthraquinone, 1,8-dihydroxyanthraquinone, 2-ethylanthraquinone, methyl-p-benzoquinone, 1,4-naphthoquinone, tetramethyl-p-benzoquinone, 2,2-dimethyl-1,3-dioxan-4,6-dione, 2-benzoylbenzoic acid, 3-benzoylpropionic acid, 5,6-dimethoxyphthalaldehydic acid, levulinic acid, methyl trans-4-oxo-2-pentenoate, phthalaldehydic acid, terephthalaldehydic acid, dibutyl maleate, dibutyl succinate, dibutyl phthalate, dicyclohexyl phthalate, diethyl acetamidomalonate, diethyl adipate, diethyl benzylmalonate, diethyl butylmalonate, diethylethoxymethylene-malonate, diethyl ethylmalonate, diethyl fumarate, diethyl glutarate, diethyl isopropylidenemalonate, diethyl maleate, diethyl malonate, diethyl methylmalonate,

diethyl oxalate, diethyl 3-oxoglutarate, diethyl phenylmalonate, diethyl phthalate, diethyl pimelate, diethyl sebacate, diethyl suberate, diethyl succinate, diisobutyl phthalate, dimethyl acetylene- dicarboxylate, dimethyl acetylsuccinate, dimethyl adipate, dimethyl 2-aminoterephthalate, dimethyl fumarate, dimethyl glutaconate, dimethyl glutarate, dimethyl isophthalate, dimethyl malonate, dimethylmethoxy-malonate, dimethyl methylenesuccinate, dimethyl oxalate, dimethyl 3-oxoglutarate, dimethyl phthalate, dimethyl succinate, dimethyl terephthalate, ethylene glycol diacetate, ethylene glycol dimethacrylate, monoethyl fumarate, monomethyl malonate, monoethyl adipate, monomethyl phthalate, monomethyl pimelate, monomethyl terephthalate, 1,2-propylene glycol diacetate, triethyl methanetricarboxylate, trimethyl 1,2,3-propanetricarboxylate, 3-acetoxy-2-cyclohexen-1-one, allyl acetoacetate, allyl cyanoacetate, benzyl acetoacetate, tert.butyl acetoacetate, butyl cyanoacetate, chlorogenic acid hemihydrate, coumarin-3-carboxylic acid, diethyl ethoxy-carbonylmethanephosphonate, dodecyl gallate, dodecyl 3,4,5-trihydroxybenzoate, (2,3-epoxypropyl) methacrylate, (2-ethoxyethyl) acetate, ethyl acetamidocyanoacetate, ethyl 2-aminobenzoate, ethyl 3-aminopyrazol-4-carboxylate, ethyl benzoxyacetate, ethyl butyrylacetate, ethyl cyanoacetate, ethyl 2-cyano-3-ethoxyacrylate, ethyl cyanoformate, ethyl 2-cyanopropionate, ethyl 3,3-diethoxypropionate, ethyl 1,3-dithian-2-carboxylate, ethyl 2-ethoxyacetate, ethyl 2-furancarboxylate, ethyl levulinate, ethyl mandelate, ethyl gallate, ethyl 2-methylactate, ethyl 4- nitrocinnamate, ethyl oxamate, ethyl 2-oxocyclohexanecarboxylate, ethyl 4-oxocyclohexane- carboxylate, ethyl 5-oxohexanoate, ethyl 2-phenylacetoacetate, ethyl 4-piperidinecarboxylate, ethyl 2-pyridinecarboxylate, ethyl 3-pyridinecarboxylate, ethyl 4-pyridinecarboxylate, ethyl thioglycolate, ethyl 3,4,5-trihydroxybenzoate, 2-hydroxyethyl methacrylate, 2-hydroxypropyl methacrylate, 3-indole acetate, 2-methoxyethyl acetate, 1-methoxy-2-propyl acetate, methyl 2- aminobenzoate, methyl 3-aminocrotonate, methyl cyanoacetate, methyl 4-cyanobenzoate, methyl 4- formylbenzoate, methyl 2-furancarboxylate, methyl isobutyrylacetate, methyl methoxyacetate, methyl 2-methoxybenzoate, methyl 3-oxopentanoate, methyl phenylglyoxylate, methyl phenyl-sulfinylacetate, methyl pivatoylacetate, methyl 3-pyridinecarboxylate, 5-nitrofurfurylidene diacetate, propyl gallate, propyl 3,4,5-trihydroxybenzoate, methyl 3-methylthiopropionate, acetamide, acetanilide, benzamide, benzanilide, N,N-diethylacetamide, N,N-dimethylformamide, N,N-diethyl-3-methyl- benzamide, diethyltoluamide, N,N-dimethylacetamide, N,N-diphenylacetamide, N-methylformamide, N-methylformanilide, N-acetylthiourea, adipic acid diamide, 2-aminobenzamide, 4-aminobenzamide, succinic acid diamide, malonic acid diamide, N,N'-methylene diacrylamide, oxalic acid diamide, pyrazine-2-carboxamide, pyridine-4-carboxamide, N,N,N',N'-tetramethylsuccinic acid diamide, N,N,N',N'-tetramethylglutaric acid diamide, acetoacetanilide, benzohydroxamic acid, cyanoacetamide, 2-ethoxybenzamide, diethyl acetamidomalonate, ethyl acetamidocyanoacetate, ethyl oxamate, hippuric acid Na salt, N-(hydroxymethyl)acrylamide, L-(-)-lactamide, 2'-nitroacetanilide, 3'- nitroacetanilide, 4'-nitroacetanilide, paracetamol, piperine, salicylanilide, 2-acetyl- γ -butyrolactone, γ -butyrolactone, ϵ -caprolactone, dihydrocoumarin, 4-hydroxycoumarin, 2-(5H)-furanone, 2,5-dihydro-5-methoxy-2-furanone, phthalide, tetrahydrofuran-2,4-dione, 2,2,6-trimethyl-1,3-dioxin-4-one, γ - valerolactone, 4-amino-1,3-dimethyluracil, barbituric acid, O-benzyloxycarbonyl-N-hydroxysuccinimide, succinimide, 3,6-dimethyl-piperazin-2,5-dione, 5,5-diphenylhydantoin, ethyl 1,3-dioxoisindoline-2-carboxylate,

Also preferred are anhydrides, such as the following:

Benzoic anhydride, benzene-1,2,4,5-tetracarboxylic acid-1,2,4,5-dianhydride, 3,3',4,4'-benzophenonetetracarboxylic anhydride, succinic anhydride, butyric anhydride, crotonic anhydride, cis-1,2-cyclohexanedicarboxylic anhydride, di-tert-butyl dicarbonate, dimethyl dicarbonate, dodecenylsuccinic anhydride, Epicon B 4400, acetic anhydride, glutaric anhydride, hexanoic anhydride, isatoic anhydride, isobutyric anhydride, isovaleric anhydride, maleic anhydride, 1,8-naphthalenedicarboxylic anhydride, 3-nitrophthalic anhydride, 5-norbornene-2,3-dicarboxylic anhydride, phthalic anhydride, 2-phenylbutyric anhydride, pivalic anhydride, propionic anhydride, cis-1,2,3,6-tetrahydrophthalic anhydride and valeric anhydride.

Particularly preferred are benzophenones such as the following:

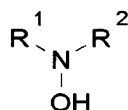
Benzophenone, 4-aminobenzophenone, 2-amino-5-chlorobenzophenone, benzophenone-2-carboxylic acid, (S)-(-)-2-(N-benzopropyl)aminobenzophenone, 4,4'-bis(dimethylamino)benzophenone, 4,4'-bis(diethylamino)benzophenone, 3,4-dimethoxybenzophenone, 4,4'-dihydroxybenzophenone, 2,4-dihydroxybenzophenone, 4-hydroxybenzophenone, 2-hydroxy-4-methoxybenzophenone, 4-methoxybenzophenone, 4,4'-dimethoxybenzophenone, 2,2',4,4'-tetrahydroxybenzophenone and 2-chlorobenzophenone.

glyoxal hydrate, 1,3,5-tris(2-hydroxyethyl)isocyanuric acid, quinalizarin and 2,4,5-trihydroxybenzamine.

APPENDIX IV

Appendix IV shows the formulas of mediators/mediation enhancers (N0-, NOH- and HNR-OH compounds) which according to the invention can be added to the enzyme component system (ECS) together with oxidoreductases, such as, for example:

Hydroxylamines (linear or cyclic, aliphatic or aromatic, heterocyclic) of general formula I)



(I)

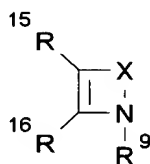
(Amended) The substituents R¹ and R², which may be the same or different, independently of one other represent one of the following groups: hydrogen, C₁-C₁₂ alkyl, carbonyl C₁-C₆ alkyl, phenyl, aryl, of which C₁-C₁₂ alkyl, carbonyl C₁-C₆ alkyl, phenyl, aryl groups may be unsubstituted or may also be substituted once or multiple times with the radical R³.

The radical R³ may represent one of the following groups: hydrogen, halogen, hydroxyl, formyl, carboxy and salts and esters thereof; amino, nitro, C₁₂-C₁₂ alkyl, C₁-C₆ alkyloxy, carbonyl C₁-C₆ alkyl, phenyl, sulfono, their esters and salts, sulfamoyl, carbamoyl, phospho, phosphono, phosphonoxy and their salts and esters. The amino, carbamoyl and sulfamoyl groups of the radical R³ may be unsubstituted or may be substituted once or two times with hydroxy, C₁-C₃ alkyl, C₁-C₃ alkoxy.

The radicals R¹ and R² can jointly form a group -B-. In that case, -B- represents one of the following groups: (-CHR⁴-)_n, (CR⁴=CH-)_m. n represents an integer from 1 to 6 and m represents an integer from 1 to 3.

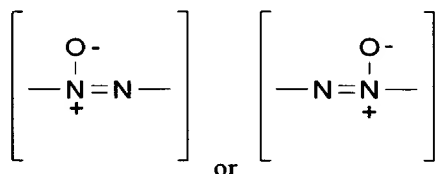
R⁴ is a substituent that is defined like R³.

such as compounds of general formula II



(II)

(Amended) X stands for one of the following groups: $(-\text{N}=\text{N}-)$, $(-\text{N}=\text{CR}^{10}-)_p$, $(-\text{CR}^{10}=\text{N}-)_p$, $(-\text{CR}^{11}=\text{CR}^{12}-)_p$,



and p is equal to 1 or 2.

The radicals R^9 to R^{12} , R^{15} and R^{16} may be the same or different and independently of one another can represent one of the following groups: hydrogen, halogen, hydroxyl, formyl, carboxyl and salts and esters thereof, amino, nitro, C_1 - C_{12} alkyl, C_1 - C_6 alkyloxy, carbonyl C_1 - C_6 alkyl, phenyl, sulfono, esters and salts thereof, sulfamoyl, carbamoyl, phospho, phosphono, phosphonooxy and their salts and esters. The amino, carbamoyl and sulfamoyl groups of the radicals R^9 to R^{12} , R^{15} and R^{16} may be unsubstituted or may also be substituted once or two times with hydroxyl, C_1 - C_3 alkyl, C_1 - C_3 alkoxy. The radicals R^{15} and R^{16} can form a common group $-\text{G}-$. $-\text{G}-$ represents one of the following groups:

$(-\text{CR}^5=\text{CR}^6-\text{CR}^7=\text{CR}^8-)$ or $(-\text{CR}^8=\text{CR}^7-\text{CR}^6=\text{CR}^5-)$.

The radicals R^5 to R^8 may be the same or different and independently of one another can represent one of the following groups: hydrogen, halogen, hydroxyl, formyl, carboxyl and salts and esters thereof, amino, nitro, C_1 - C_{12} alkyl, C_1 - C_6 alkyloxy, carbonyl, C_1 - C_6 alkyl, phenyl, sulfono, esters and salts thereof, sulfamoyl, carbamoyl, phospho, phosphono, phosphonooxy and their salts and esters. The amino, carbamoyl and sulfamoyl groups of the radicals R^5 to R^8 may be unsubstituted or may also be

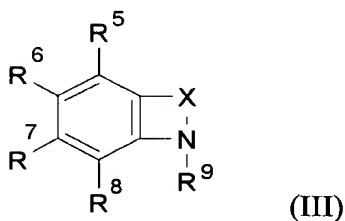
substituted once or two times with hydroxy, C₁-C₃ alkyl, C₁-C₃ alkoxy.

The C₁-C₁₂ alkyl, C₁-C₆ alkyloxy, carbonyl C₁-C₆ alkyl, phenyl and aryl groups of radicals R⁵ to R⁸ may be unsubstituted or be substituted one or two times with the radical R¹⁸. The radical R¹⁸ can represent one of the following groups: hydrogen, halogen, hydroxyl, formyl, carboxyl and their salts and esters, amino, nitro, C₁-C₁₂ alkyl, C₁-C₆ alkyloxy, carbonyl C₁-C₆ alkyl, phenyl, aryl, and their esters and salts.

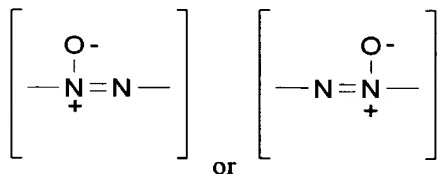
The carbamoyl, sulfamoyl and amino groups of the radical R¹⁸ may be unsubstituted or may also be substituted once or two times with the radical R¹⁹.

The radical R¹⁹ may represent one of the following groups: hydrogen; hydroxyl, formyl, carboxyl and their salts and esters; amino, nitro, C₁-C₁₂ alkyl, C₁-C₆ alkyloxy, carbonyl C₁-C₆ alkyl, phenyl and aryl.

such as compounds of general formula III:



(Amended) X stands for one of the following groups: (-N=N-), (-N=CR¹⁰-)_p, (-CR¹⁰=N-)_p, (-CR¹¹=CR¹²-)_p

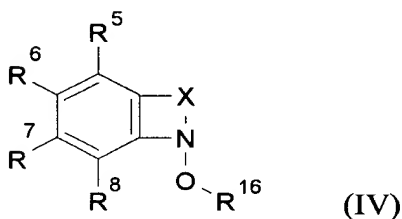


and p is equal to 1 or 2.

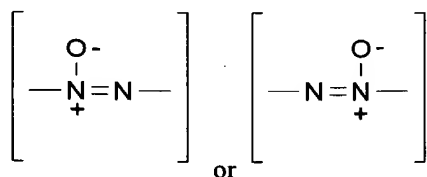
The radicals R⁵ to R¹² may be the same or different and independently of one another can represent one of the following groups: hydrogen, halogen, hydroxyl, formyl, carboxyl and salts and esters thereof, amino, nitro, C₁-C₁₂ alkyl, C₁-C₆ alkyloxy, carbonyl C₁-C₆ alkyl, phenyl, aryl, sulfono esters and salts thereof, sulfamoyl,

carbamoyl, phospho, phosphono, phosphonoxy and their salts and esters. The amino, carbamoyl and sulfamoyl groups of the radicals R^5 to R^{12} may be unsubstituted or may also be substituted once or two times with hydroxyl, C_1 - C_3 alkyl, C_1 - C_3 alkoxy. The C_1 - C_{12} alkyl, C_1 - C_6 alkyloxy, carbonyl C_1 - C_6 alkyl, phenyl, aryl, aryl C_1 - C_6 alkyl groups of radicals R^5 to R^{12} may be unsubstituted or substituted once or two times with the radical R^{13} . The radical R^{13} can represent one of the following groups: hydrogen, halogen, hydroxyl, formyl, carboxyl and their salts and esters; amino, nitro, C_1 - C_{12} alkyl, C_1 - C_6 alkyloxy, carbonyl C_1 - C_6 alkyl, phenyl, aryl, sulfono, sulfeno, sulfinio, and their esters and salts. The carbamoyl, sulfamoyl, amino groups of the radical R^{13} may be unsubstituted or may also be substituted once or two times with the radical R^{14} . The radical R^{14} may represent one of the following groups: hydrogen, hydroxyl, formyl, carboxyl and their salts and esters; amino, nitro, C_1 - C_{12} alkyl, C_1 - C_6 alkyloxy, carbonyl C_1 - C_6 alkyl, phenyl or aryl.

such as compounds of general formula IV:



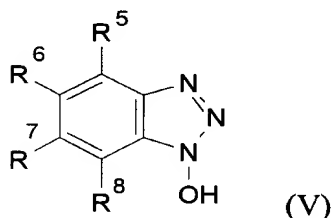
(Amended) X stands for one of the following groups: $(-N=N-)$, $(-N=CR^{10}-)_m$, $(-CR^{10}=N-)_m$, $(-CR^{11}=CR^{12}-)_m$



and m is equal to 1 or 2.

For the radicals R^5 to R^8 and R^{10} to R^{12} what has been said above applies.
 R^{17} can be hydrogen, C_1 - C_{10} alkyl, C_1 - C_{10} carbonyl, of which C_1 - C_{10} alkyl and C_1 - C_{10} carbonyl can be unsubstituted or mono- or polysubstituted with a radical R^{18} , which is defined like R^3 .

such as compounds, namely derivatives, of 1-hydroxybenzotriazole and the tautomeric benzotriazole 1-oxide, as well as the esters and salts thereof, particularly compounds of formula V :

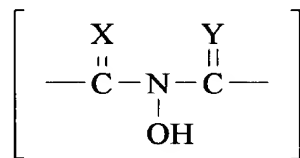


(Amended) The radicals R^5 to R^8 may be the same or different and independently of one another can represent one of the following groups: hydrogen, halogen, hydroxyl, formyl, carboxyl and salts and esters thereof, amino, nitro, C_1 - C_{12} alkyl, C_1 - C_6 alkyloxy, carbonyl C_1 - C_6 alkyl, phenyl, sulfono esters and salts thereof, sulfamoyl, carbamoyl, phospho, phosphono, phosphonooxy and their salts and esters. The amino, carbamoyl and sulfamoyl groups of the radicals R^5 to R^8 may be unsubstituted or may also be substituted once or two times with hydroxyl, C_1 - C_3 alkyl, C_1 - C_3 alkoxy. The C_1 - C_{12} alkyl, C_1 - C_6 alkyloxy, carbonyl C_1 - C_6 alkyl, phenyl or aryl groups of radicals R^5 to R^8 may be unsubstituted or may also be substituted one or mono- or polysubstituted with the radical R^{18} . The radical R^{18} can represent one of the following groups: hydrogen, halogen, hydroxyl, formyl, carboxyl and their salts and esters; amino, nitro, C_1 - C_{12} alkyl, C_1 - C_6 alkyloxy, carbonyl C_1 - C_6 alkyl, phenyl, aryl, sulfono, sulfeno, sulfino, and their esters and salts. The carbamoyl, sulfamoyl, amino groups of the radical R^{18} may be unsubstituted or may also be substituted once or two times with the radical R^{19} . The radical R^{19} may represent one of the following groups: hydrogen, hydroxyl, formyl, carboxyl and their salts and esters, amino, nitro, C_1 - C_{12} alkyl, C_1 - C_6 alkyloxy, carbonyl C_1 - C_6 alkyl, phenyl or aryl.

such as, for example, the following compounds:

1 -hydroxybenzotriazole
1 -hydroxybenzotriazole, sodium salt
1 -hydroxybenzotriazole, potassium salt
1 -hydroxybenzotriazole, lithium salt
1 -hydroxybenzotriazole, ammonium salt
1 -hydroxybenzotriazole, calcium salt
1 -hydroxybenzotriazole, magnesium salt
1 -hydroxybenzotriazole-6-sulfonic acid, monosodium salt
1 -methoxy-1 H-benzotriazole
1 -acetoxy- 1 H-benzotriazole
1 -hydroxy-(4,5-f)-dioxolo-1 H-benzotriazole
1 -hydroxy-6-methyl-3H-benzotriazole
1 -hydroxy-6-nitro-1 H-benzotriazole
1 -hydroxy-5,6-dimethyl-1 H-benzotriazole
1 -hydroxy-6-methoxy-1 H-benzotriazole
1 -hydroxy-5,6-dimethoxy-1 H-benzotriazole
1 -hydroxy-1 H-benzotriazole-6-carboxylic acid
1,5-dihydroxy-1 H-benzotriazole
1 -hydroxy-1 H-benzotriazole-6-sulfonic acid hydrazide
1 -hydroxy-1 H-benzotriazole-6-carboxamide
1 -hydroxy-5-methoxy-1 H-benzotriazole
6-amino-1 -hydroxy-1 H-benzotriazole
6-amino-5-methoxy-1 H-benzotriazole
6-chloro- 1 -hydroxy- 1 H-benzotriazole
6-acetamido-1 -hydroxy-1 H-benzotriazole
1 -hydroxy- 1 H-benzotriazole-6-carboxylic acid ethyl ester
1 -hydroxy-4-nitro-1 H-benzotriazole
4-chloro-1 -hydroxy-1 H-benzotriazole
1 -hydroxy-6-tert. butyl- 1 H-benzotriazole
6-cyclohexyl-1 -hydroxy-1 H-benzotriazole
6-isopropyl-1 -hydroxy-1 H-benzotriazole
1 -hydroxy-6-phenyl-1 H-benzotriazole
3-methyl-3H-benzotriazole 1 -oxide
2-phenyl-2H-benzotriazole 1 -oxide

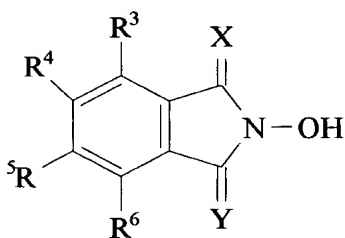
such as compounds of general formula A (cyclic N-hydroxy compounds):



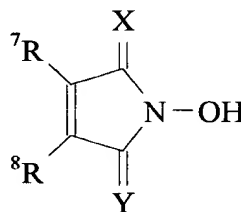
(A)

(Amended) as well as their salts, ethers or ester, in which X and Y are the same or different and stand for O, S or NR¹, in which R¹ stands for hydrogen, hydroxyl, formyl, carbamoyl, or sulfono radical, or ester or salt of the sulfono radical, sulfamoyl, nitro, amino, phenyl, aryl C₁-C₅ alkyl, C₁-C₁₂ alkyl, C₁-C₅ alkoxy, C₁-C₁₀ carbonyl, carbonyl C₁-C₆ alkyl, phospho, phosphono or phosphonooxy radical, or ester or salt of the phosphonooxy radical, and carbamoyl, sulfamoyl, amino and phenyl radicals may be unsubstituted or substituted once or multiple times with a radical R², and the aryl C₁-C₅ alkyl, C₁-C₁₂ alkyl, C₁-C₅ alkoxy, C₁-C₁₀ carbonyl, carbonyl C₁-C₆ alkyl radicals may be saturated or unsaturated, branched or unbranched, and substituted once or multiple times with a radical R² and R² is the same or different and stands for hydroxyl, formyl, or carboxyl radical, ester or salt of the carboxy radical, carbamoyl, sulfono ester or salt of the sulfono radical, sulfamoyl, nitro, amino, phenyl, C₁-C₅ alkyl, C₁-C₅ alkoxy radical.

Such as compounds of general formula VI, VII, VIII or IX:

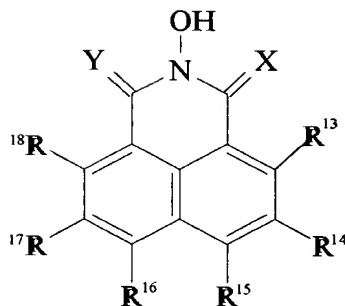
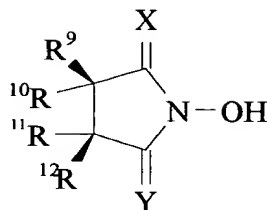


VI



VII

VIII

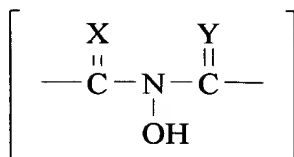


IX

(Amended) in which X, Y have the meanings already given and the radicals R³ to R¹⁸ are the same or different and stand for halogen radical, carboxy radical, salt or ester of a carboxy radical, or the meaning given for R¹,

in which R⁹ and R¹⁰, or R¹¹ and R¹², must not at the same time stand for a hydroxyl or amino radical and optionally two at a time of the substituents R³ to R⁶, R⁷ to R⁸, R⁹ to R¹², R¹³ to R¹⁸ can be linked together into a ring -B-, in which -B- has one of the following meanings:

(-CH=CH)-_n, where n = 1 to 3, -CH=CH-CH=N-, or



(A)

(Amended) and in which optionally the radicals R^9 to R^{12} may also be linked to one another by one or two bridge elements -Q-, in which -Q- may be the same or different and can have the following meanings: -O-, -S-, CH_2 -, $-\text{CR}^{19}=\text{CR}^{20}-$, in which R^{19} and R^{20} are the same or different and have the same meaning as R^3 , and in which X and Y stand for O or S.

for example, compounds such as:

N-hydroxyphthalimide and optionally substituted N-hydroxyphthalimide derivatives, N-hydroxymaleimide and optionally substituted N-hydroxymaleimide derivatives, N-hydroxynaphthalimide and optionally substituted N-hydroxynaphthalimide derivatives, N-hydroxysuccinimide and optionally substituted N-hydroxysuccinimide derivatives, **such as, for example:**

N-hydroxyphthalimide, N-hydroxybenzene-1,2,4-tricarboximide,
N,N'-dihydroxypyromellitic acid diimide,
N,N'-dihydroxybenzophenone-3,3',4,4'-tetracarboxylic acid diimide,
for example, (formula VII):

N-hydroxymaleimide,
pyridine-2,3-dicarboxylic acid N-hydroximide

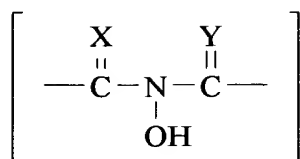
for example (formula VIII):

N-1-hydroxysuccinimide, N-1-hydroxytartarimide,
N-hydroxy-5-norbornene-2,3-dicarboximide,
exo-N-hydroxy-7-oxabicyclo[2.2.1]-5-heptene-2,3-dicarboximide,
N-hydroxy-cis-cyclohexane-1,2-dicarboximide,
N-hydroxy-cis-4-cyclohexene-1,2-dicarboximide

for example (formula IX):

N-hydroxynaphthalimide sodium salt

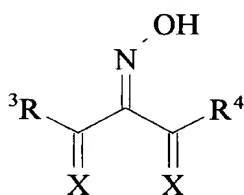
for example (six-membered ring of formula A):



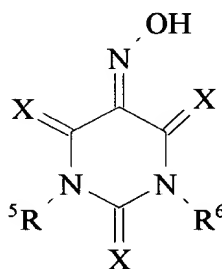
(A)

N-hydroxyglutarimide

such as compounds of general formula X or XI (oximes):



X



XI

(Amended) and their salts, ethers or esters, in which X is the same or different and stands for O, S or NR¹, in which R¹ stands for hydrogen, hydroxyl, formyl, carbamoyl, or sulfono radical, or ester or salt of the sulfono radical, sulfamoyl, nitro, amino, phenyl, acryl C₁-C₅ alkyl, C₁-C₁₂ alkyl, C₁-C₅ alkoxy, C₁-C₁₀ carbonyl, carbonyl C₁-C₆ alkyl, phospho, phosphono or phosphonooxy radical, or ester or salt of the phosphonooxy radical, in which carbamoyl, sulfamoyl, amino and phenyl radicals may be unsubstituted or substituted once or multiple times with a radical R², and the aryl C₁-C₅ alkyl, C₁-C₁₂ alkyl, C₁-C₅ alkoxy, C₁-C₁₀ carbonyl, carbonyl C₁-C₆ alkyl radicals may be saturated or unsaturated, branched or unbranched, and substituted once or multiple times with a radical R², and R² is the same or different and stands for hydroxy, formyl, or carboxyl radical, ester or salt of the carboxyl radical, carbamoyl, sulfono ester or salt of the sulfono radical, sulfamoyl, nitro, amino, phenyl, C₁-C₅ alkyl, C₁-C₅ alkoxy radical, and the radicals R³ and R⁴ are the same or different and stand for halogen, carboxyl radical, ester or salt of the carboxyl radical, or have the meanings given for R¹, or are linked together into a ring (-CR⁷R⁸)_n, where n is equal to 2, 3 or 4, and R⁵ and R⁶ have the

for example (formula X):

for example (formula XI):

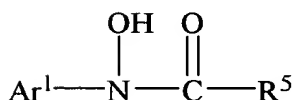
(Amended) such as compounds from the class of N-aryl-N-hydroxyamides of general formula XII, XIII and XIV: (XIVa, XIVb, XIVc, XIVd and XIVE:)



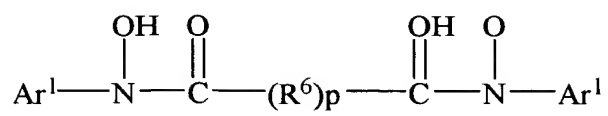
A is a monovalent homoaromatic or heteroaromatic monocyclic or bicyclic group and D is a divalent homoaromatic or heteroaromatic monocyclic or bicyclic group, these aromatics possibly being substituted with one or more equal or different R1 groups selected from the group consisting of halogen or a hydroxyl, formyl, cyano, carbamoyl

or carboxyl group, an ester or salt of the carboxyl group, a sulfo group, an ester or salt of the sulfo group, or sulfamoyl, nitro, nitroso, amino, phenyl, aryl-C₁-C₅ alkyl, C₁-C₁₂ alkyl, C₁-C₅ alkoxy, C₁-C₁₀ carbonyl, carbonyl-C₁-C₆ alkyl, phospho, phosphono or phosphonoxy group, or an ester or salt of the phosphonoxy group, and the carbamoyl, sulfamoyl, amino and phenyl groups are unsubstituted or substituted with one or several R² groups, and the aryl-C₁-C₅ alkyl, C₁-C₁₂ alkyl, C₁-C₅ alkoxy, C₁-C₁₀ carbonyl, carbonyl-C₁-C₆ alkyl groups are saturated or unsaturated, branched or unbranched, and can be substituted with one or more R² groups, with the R₂ groups being equal or different and denoting a hydroxyl, formyl, cyano or carboxyl group, an ester or a salt of the carboxyl group, or a carbamoyl, sulfonyl, sulfamoyl, nitro, nitroso, amino, phenyl, C₁-C₅ alkyl, C₁-C₅ alkoxy or C₁-C₅ alkylcarbonyl group and pairs of R¹ or R² groups are joined by a (-CR³R⁴-)_m link, with m equal to 1, 2, 3 or 4 and R³ and R⁴ are equal or different and denote a carboxyl group, an ester or salt of a carboxyl group, a phenyl, C₁-C₅ alkyl, C₁-C₅ alkoxy or C₁-C₅ alkylcarbonyl group and one or more of the nonadjacent (-CR³R⁴-) groups can be replaced with oxygen, sulfur or an optionally C₁-C₅ alkyl group-substituted imino group, and two adjacent (-CR³R⁴-) groups can be replaced by a (-CR³=R⁴-) group, and B denotes a monovalent acid radical, in the amide form, of an acid selected from the group consisting of carboxylic acids with up to 20 carbon atoms, carbonic acid, half-esters of carbonic or carbamic acid, sulfonic acid, phosphonic acid, phosphoric acid, monoesters of phosphoric acid or diesters of phosphoric acid. And C denotes a divalent acid radical, in the amide form, of an acid selected from the group consisting of monocarboxylic and dicarboxylic acids with up to 20 carbon atoms, carbonic acids, sulfonic acids, phosphonic acids, phosphoric acids and monoesters of phosphoric acid.

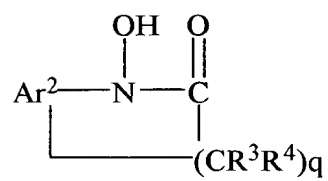
(Amended) such as compounds from the class of N-aryl-N-hydroxyamides of general formula (XII, XIII and XIV:) XIVa, XIVb, XIVc, XIVd and XIVE:



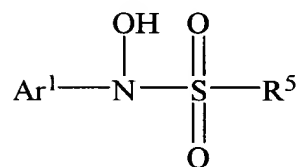
XIVa



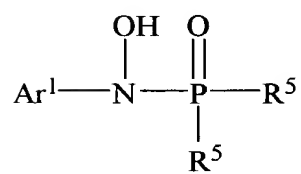
XIVb



XIVc



XIVd



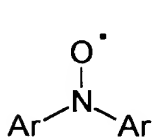
XIVe

(Amendends) and the salts, ethers and esters thereof, wherein Ar^1 denotes a homoaromatic or heteroaromatic monocyclic aryl group and Ar^2 is a divalent homoaromatic or heteroaromatic monocyclic aryl group, possibly substituted with one or more equal or different R^7 groups selected from among hydroxy, cyano or carboxyl groups, or esters or salts of the carboxyl group, or the sulfo group or esters or salts of the sulfo group, or the nitro, nitroso, amino, C_1-C_{12} alkyl, C_1-C_5 alkoxy, C_1-C_{10} carbonyl, carbonyl- C_1-C_6 alkyl group, wherein the amino groups are unsubstituted or substituted with one or more R^8 groups and the C_1-C_{12} alkyl, C_1-C_5 alkoxy, C_1-C_{10} carbonyl, carbonyl- C_1-C_6 alkyl groups are saturated or unsaturated, branched or unbranched and possibly are substituted with one or more R^8 groups, said groups being equal or different and denoting a hydroxyl or carboxyl group, an ester or salt of the carboxyl group, a sulfo, nitro, amino, C_1-C_5 alkyl, C_1-C_5 alkoxy or C_1-C alkylcarbonyl group, and pairs of R^7 groups are connected by a $(-CR^3R^4-)_m$ link, with m equal to 0, 1, 2, 3 or 4, and R^3 and R^4 have the aforesaid meaning, and one or more of the adjacent $(-CR^3R^4-)$ groups are possibly substituted with oxygen, sulfur or an imino group optionally substituted with a C_1-C_5 alkyl group, and two adjacent $(-CR^3R^4-)$ groups are substituted with a $(-CR^3=R^4-)$ group, and R^5 denotes equal or different monovalent groups selected from among phenyl, aryl- C_1-C_5 -alkyl, C_1-C_{12} alkyl, C_1-C_5 alkoxy and C_1-C_{10} carbonyl groups, the phenyl groups possibly being unsubstituted or substituted with one or more R^9 groups, and the aryl- C_1-C_5 alkyl, C_1-C_{12} alkyl, C_1-C_5 -alkoxy and C_1-C_{10} carbonyl groups are saturated or unsaturated, branched or unbranched and possibly substituted with one or more R^6 groups, wherein the R^9 groups are equal or different and denote a hydroxyl, formyl, cyano or carboxyl group or an ester or salt of the carboxyl group, or a carbamoyl, sulfo, sulfamoyl, nitro, nitroso, amino, phenyl, C_1-C_5 alkyl or C_1-C_{12} alkoxy group, and R^6 is a divalent group selected from among the ortho, meta and para-phenylene, aryl- C_1-C_5 alkyl, C_1-C_{12} alkylene and C_1-C_5 alkylendioxy groups, the phenylene groups possibly being unsubstituted or substituted with one or more R^9 groups, and the aryl- C_1-C_5 alkyl, C_1-C_{12} alkyl and C_1-C_5 alkoxy groups possibly being saturated or unsaturated, branched or unbranched and possibly substituted with one or more R^9 groups, and wherein p stands for 0 or 1 and q stands for an integer of 1 to 3.

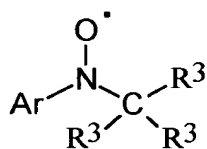
Preferably, Ar^1 denotes a phenyl group and Ar^2 an ortho-phenylene group, Ar^1 possibly being substituted with up to five and Ar^2 with up to four equal or different groups selected from among C_1-C_3 alkyl or C_1-C_3 alkylcarbonyl groups or carboxyl groups or sulfo groups, or esters or salts of the sulfo group, or a hydroxyl, cyano, nitro, nitroso or amino group, wherein the amino groups are possibly substituted with two different groups selected from among the hydroxyl and C_1-C_3 alkylcarbonyl groups. R^5 preferably denotes a monovalent group selected from among hydrogen and the phenyl, C_1-C_{12} alkyl and C_1-C_3 alkoxy groups, wherein the C_1-C_{12} alkyl groups and the

C₁-C₃ alkoxy groups are saturated or unsaturated, branched or unbranched.
R⁶ preferably denotes divalent groups selected from among the ortho- or para-phenylene, C₁-C₁₂ alkylene, C₁-C₅ alkylendioxy groups, wherein the aryl-C₁-C₅ alkyl, C₁-C₁₂ alkyl and C₁-C₅ alkoxy groups are saturated or unsaturated, branched or unbranched and are possibly substituted with one or more R⁵ groups.
Preferably, R⁹ denotes a carboxyl group, an ester or salt of the carboxyl group or a carbamoyl, phenyl or C₁-C₃ alkoxy group.

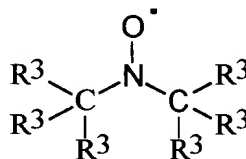
such as compounds of general formula XV, XVI and XVII (nitroxyl radicals/nitroxides):



XV



XVI

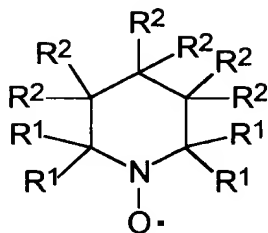


XVII

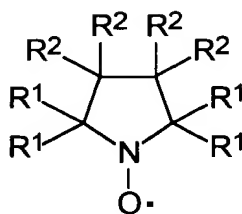
(Amended) wherein Ar denotes a monovalent homoaromatic or heteroaromatic monocyclic or bicyclic group and wherein these aromatics are possibly substituted with one or more equal or different R₁ groups selected from among the groups consisting of halogen, formyl, cyano, carbamoyl and carboxyl groups, esters and salts of the carboxyl group, or the sulfo group or esters or salts of the sulfo group, or sulfamoyl, nitro, nitroso, amino, phenyl, aryl-C₁-C₅ alkyl, C₁-C₁₂ alkyl, C₁-C₅ alkoxy, C₁-C₁₀ carbonyl, carbonyl-C₁-C₆ alkyl, phospho, phosphono or phosphonoxy group or an ester or salt of the phosphonoxy group, and wherein the phenyl, carbamoyl and sulfamoyl groups can be unsubstituted or substituted with one or more R² groups, the amino group possibly

being substituted with one or two R^2 groups, and the aryl- C_1 - C_5 alkyl, C_1 - C_{12} alkyl, C_1 - C_5 alkoxy, C_1 - C_{10} carbonyl and carbonyl- C_1 - C_6 alkyl groups being saturated or unsaturated, branched or unbranched and possibly substituted with one or more R^2 groups, wherein the R^2 groups are present once or several times and are equal or different and denote a hydroxyl, formyl or cyano group or carboxyl group or an ester or salt of the carboxyl group, or a carbamoyl, sulfo, sulfamoyl, nitro, nitroso, amino, phenyl, C_1 - C_5 alkyl, C_1 - C_5 alkoxy or C_1 - C_5 alkylcarbonyl group, and wherein the R^3 groups are present once or several times and are equal or different and denote a halogen or a hydroxyl, mercapto, formyl, cyano, carbamoyl or carboxyl group, or an ester or salt of the carboxyl group, or a sulfo group, or an ester or salt of the sulfo group, or a sulfamoyl, nitro, nitroso, amino, phenyl, aryl- C_1 - C_5 alkyl, C_1 - C_{12} alkyl, C_1 - C_5 alkoxy, C_1 - C_{10} carbonyl, carbonyl- C_1 - C_6 alkyl, phospho, phosphono or phosphonoxy group or an ester or salt of the phosphonoxy group and wherein R^3 in the case of stable bicyclic nitroso radicals (structure N) stands also for hydrogen and wherein the carbamoyl, sulfamoyl, amino, mercapto and phenyl groups are unsubstituted or substituted with one or more R^4 groups and the aryl- C_1 - C_5 alkyl, C_1 - C_{12} alkyl, C_1 - C_5 alkoxy, C_1 - C_{10} carbonyl and carbonyl- C_1 - C_6 alkyl groups are saturated or unsaturated, branched or unbranched and are possibly substituted with one or more R^4 groups, wherein the R^4 groups are equal or different and denote a hydroxyl, formyl, cyano or carboxyl group or an ester or salt of the carboxyl group, or a carbamoyl, sulfo, sulfamoyl, nitro, nitroso, amino, phenyl, C_1 - C_5 alkyl, C_1 - C_5 alkoxy or C_1 - C_5 alkylcarbonyl group, and pairs of R^3 or R^4 groups can be joined by a $(-R^5R^6-)_m$ link with m equal to 0, 1, 2, 3 or 4, and R^5 and R^6 are equal or different and denote halogen, a carboxyl group, an ester or salt of the carboxyl group, or a carbamoyl, sulfamoyl, phenyl, benzoyl, C_1 - C_5 alkyl, C_1 - C_5 alkoxy or C_1 - C_5 alkylcarbonyl group, and one or more nonadjacent $(-R^5R^6-)$ groups can be replaced with oxygen, sulfur or an optionally C_1 - C_5 -alkyl-substituted imino group, and two adjacent $(-R^5R^6-)$ groups can be replaced with a $(-R^5=R^6-)$, $(-CR^5=N-)$ or $(CR^5=N(O)-)$ group.

such as compounds of general formula XVII a and XVII b (nitroxyl radicals):



XVIIa

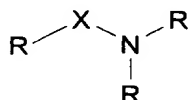


XVIIb

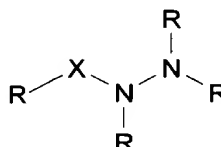
(Amended) wherein the R^1 groups are equal or different and denote phenyl, aryl- C_1-C_5 -alkyl, C_1-C_{12} alkyl, C_1-C_5 alkoxy, C_1-C_{10} carbonyl or carbonyl- C_1-C_6 alkyl groups and the phenyl groups are unsubstituted or substituted with one or more R^3 groups, and the aryl- C_1-C_5 alkyl, C_1-C_{12} alkyl, C_1-C_5 alkoxy, C_1-C_{10} carbonyl or carbonyl- C_1-C_6 alkyl groups are saturated or unsaturated, branched or unbranched and are possibly substituted with one or more R^3 groups, wherein the R^3 groups can be present once or several times, and are equal or different denoting a hydroxyl, formyl or carboxyl group or an ester or salt of the carboxyl group, or a carbamoyl, sulfo, sulfamoyl, viro, nitroso, amino, phenyl, benzoyl, C_1-C_5 alkyl, C_1-C_5 alkoxy or C_1-C_5 alkylcarbonyl group, and the R^2 groups are present once or several times and are equal or different, denoting hydrogen or a hydroxyl, mercapto, formyl, cyano, carbamoyl or carboxyl group or an ester or salt of the carboxyl group, or a sulfo group or an ester or salt of the sulfo group, or a sulfamoyl, nitro, nitroso, amino, phenyl, aryl- C_1-C_5 alkyl, C_1-C_{12} alkyl, C_1-C_5 alkoxy, C_1-C_{10} carbonyl, carbonyl- C_1-C_6 alkyl, phospho, phosphono or phosphonoxy group or an ester or salt of the phosphonoxy group, and wherein the carbamoyl, sulfamoyl, amino, mercapto and phenyl groups are unsubstituted or substituted with one or more R^3 groups, and the aryl- C_1-C_5 alkyl, C_1-C_{12} alkyl, C_1-C_5 alkoxy, C_1-C_{10} carbonyl or carbonyl- C_1-C_6 alkyl groups are saturated or unsaturated, branched or unbranched and possibly are substituted with one or more R^3 groups, and one ($-CR^2R^2-$) group can be replaced with oxygen, an optionally C_1-C_5 alkyl-substituted imino group, a hydroximino group, a carbonyl function or an optionally mono or disubstituted vinylidene function, and two adjacent ($-CR^2R^2-$) groups can be

replaced with a $(-CR^2=R^2-)$, $(-CR^2=N-)$ or $(-CR^2=N(O)-)$ group.

such as compounds of general formula XVIIIa (amides) and XVIII b (hydrazides):



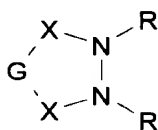
XVIIIa



XVIII b

(Amended) wherein X denotes C=O or O=S=O (carboxamides or sulfonamides). The R groups can be equal or different and independently of each can stand for hydrogen, alkyl, aryl or acyl groups (carboxylic acid or sulfonic acid groups).

such as compounds of general formula XVIII c (cyclic hydrazides):



XVIII c

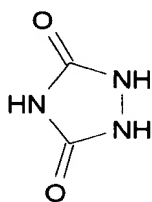
(Amended) wherein X stands for C=O or O=S=O (cyclic hydrazides of dicarboxylic acids or disulfonic acids).

The R groups can be equal or different and independently of each other represent hydrogen, alkyl, aryl or acyl groups.

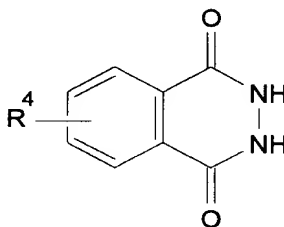
G denotes the following atoms or atomic groups: CH_2 , $\text{CH}_2\text{-CH}_2$, $\text{CHR}^1\text{-CHR}^1$, CH=CH , $\text{CR}^2\text{-CR}^2$, NH , NR^3 , C=O , ortho- C_6H_4 (ortho-substituted phenyl group), ortho- C_{10}H_6 (ortho-substituted naphthyl group), wherein the R^1 to R^3 groups can be equal or

different and independently of each other denote hydrogen, alkyl, aryl, or acyl groups.

such as urazoles (formula XVIII d) and phthalhydrazides (formula XVIII e):



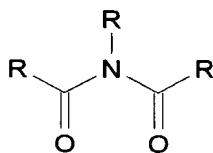
XVIII d



XVIII e

(Amended) wherein R⁴ denotes hydrogen, alkyl, alkoxy, carboxyl, nitro or amino groups. The R groups are equal or different and independently of each other stand for hydrogen, alkyl or aryl.

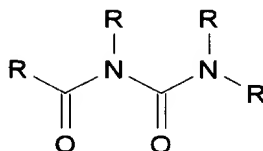
such as compounds of general formula XIX (imides):



XIX

(Amended) The R groups are equal or different and independently of each other denote hydrogen, alkyl, aryl, acyl or amino groups.

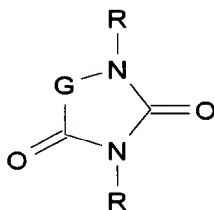
such as compounds of general formula XIXa (imides):



XIX a

(Amended) The R groups are equal or different and independently of each denote hydrogen, alkyl, aryl, acyl or amino groups.

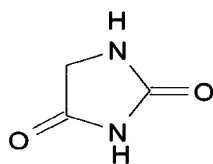
such as compounds of general formula XIXb (cyclic imides):



XIX b

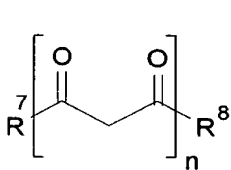
(Amended) The R groups are equal or different and independently of each other denote hydrogen, alkyl, aryl, acyl or amino groups, group G representing one of the following atoms or atomic groups: CH₂, CHR¹, CHR¹R², CH=CH, CR³-CR⁴, NH, NR⁵, C=O or O, with R¹ to R⁵ being equal or different and independently of each other denoting hydrogen, alkyl, aryl, alkoxy or carboxyl groups.

such as compounds of general formula XIXc (hydantoin derivatives):

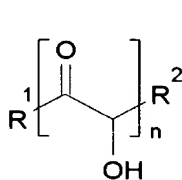


XIX c

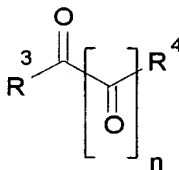
such as compounds of general formula XX, such as α -hydroxycarbonyl compounds of general formula XXa, α -dicarbonyl compounds of general formula XXb, β -hydroxycarbonyl compounds of general formula XXc and β -dicarbonyl compounds of general formula XXd:



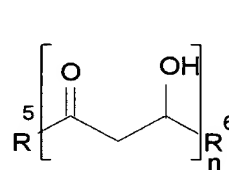
XXa



XX b



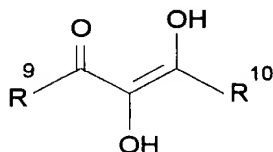
XX c



XX d

(Amended) wherein the R^1 to R^8 groups independently of each other denote one of the following atoms or atomic groups: hydrogen, halogen, alkyl, alkyloxy, aryl, aryloxy, hydroxy, oxo, formyl, thioxo, mercapto, alkylthio, sulfeno, sulfino, sulfo, sulfamoyl, amino, imino, amido, amidino, hydroxycarbamoyl, hydroximino, nitroso, nitro or hydrazono group, with R^1 and R^2 ; R^3 and R^4 ; R^5 and R^6 ; and R^7 and R^8 possibly combining to form a single group, and wherein n is greater than or equal to 1.

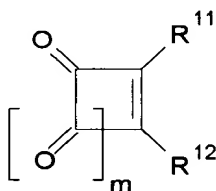
such as compounds of general formula XXI (linear compounds with double bonds/enols):



XXI

(Amended) wherein R^9 and R^{10} independently of each other denote one of the following atoms or atomic groups: hydrogen, halogen, alkyl, alkyloxy, aryl, aryloxy, hydroxy, oxo, formyl, thioxo, mercapto, alkylthio, sulfeno, sulfino, sulfo, sulfamoyl, amino, imino, amido, amidino, hydroxycarbamoyl, hydroximino, nitroso, nitro or hydrazono, with R^9 and R^{10} possibly combining to form a single group.

such as compounds of general structure XXII (cyclic compounds, groups not OH, derivatives of squaric acid, OH group derivatized):



XXII

(Amended) wherein the R^{11} and R^{12} group independently of each other denote one of the following atoms or atomic groups: hydrogen, halogen, alkyl, alkyloxy, aryl, aryloxy, hydroxy, oxo, formyl, thioxo, mercapto, alkylthio, sulfeno, sulfino, sulfo, sulfamoyl, amino, imino, amido, amidino, hydroxycarbamoyl, hydroximino, nitroso, nitro or hydrazono, and m is greater than or equal to 0.

Particularly preferred are cyclic oxocarbons of the general empirical formula: $H_2C_xO_x$ and their anions having general formula $C_xO_x^{2-}$, wherein x is greater than or equal to 3.

such as, for example:

deltic acid, squaric acid, croconic acid and rhodizonic acid

(Amended) *The formula descriptions (groups/R ... are also given in the German Published, Non Examined Patent Application DE 197 19 857.0. and in the US Patent Application Serial No. 09/029,401

APPENDIX IVa

Appendix IVa shows compounds which can be added to the enzyme component system (ECS) of the invention as mediation enhancers, primarily as additives together with mediators and oxidoreductases:

Aliphatic ethers and aryl-substituted alcohols, such as:

2,3-dimethoxybenzyl alcohol, 3,4-dimethoxybenzyl alcohol, 2,4-dimethoxybenzyl alcohol, 2,6-dimethoxybenzyl alcohol, homovanillic alcohol, ethylene glycol monophenyl ether, 2-hydroxybenzyl alcohol, 4-hydroxybenzyl alcohol, 4-hydroxy-3-methoxybenzyl alcohol, 2-methoxybenzyl alcohol, 2,5-dimethoxybenzyl alcohol, 3,4-dimethoxybenzylamine, 2,4-dimethoxybenzylamine hydrochloride, veratryl alcohol, coniferyl alcohol.

olefins (alkenes), for example:

2-allylphenol, 2-allyl-6-methylphenol, allylbenzene, 3,4-dimethoxypropenylbenzene, p-methoxystyrene, 1-allylimidazole, 1-vinylimidazole, styrene, stilbene, allyl phenyl ether, benzyl cinnamate, methyl cinnamate, 2,4,6-triallyloxy-1,3,5-triazine, 1,2,4-trivinylcyclohexane, 4-allyl-1,2-dimethoxybenzene, 4-tert. benzoic acid vinyl ester, squalene, benzoin allyl ether, cyclohexene, dihydropyran and N-benzylcinnamanilide.

Phenol ethers, such as:

2,3-dimethoxybenzyl alcohol, 3,4-dimethoxybenzyl alcohol, 2,4-dimethoxybenzyl alcohol, 2,6-dimethoxybenzyl alcohol, homovanillic alcohol, 4-hydroxybenzyl alcohol, 4-hydroxy-3-methoxybenzyl alcohol, 2-methoxybenzyl alcohol, 2,5-dimethoxybenzyl alcohol, 3,4-dimethoxybenzylamine, 2,4-dimethoxybenzylamine hydrochloride, veratryl alcohol, coniferyl alcohol, veratrol, anisole.

Carbonyl compounds, such as:

4-aminobenzophenone, 4-acetylbiphenylbenzophenone, benzil, benzophenone hydrazone, 3,4-dimethoxybenzaldehyde, 3,4-dimethoxybenzoic acid, 3,4-dimethoxybenzophenone, 4-dimethylaminobenzaldehyde, 4-acetylbiphenyl hydrazone,

benzophenone 4-carboxylic acid, benzoylacetone, bis(4,4'-dimethylamino)benzophenone, benzoin, benzoin oxime, N-benzoyl-N-phenylhydroxylamine, 2-amino-5-chlorobenzophenone, 3-hydroxy-4-methoxybenzaldehyde, 4-methoxybenzaldehyde, anthraquinone-2-sulfonic acid, 4-methylaminobenzaldehyde, benzaldehyde, benzophenone-2-carboxylic acid, 3,3',4,4'-benzophenonetetracarboxylic dianhydride, (S)-(-)-2-(N-benzylpropyl)-aminobenzophenone, benzylphenylacetanilide, N-benzylbenzanilide, 4,4'-bis(dimethylamino)thiobenzophenone, 4,4'-bis(diacetylamino)benzophenone, 2-chlorobenzophenone, 4,4'-dihydroxybenzophenone, 2,4-dihydroxybenzophenone, 3,5-dimethoxy-4-hydroxybenzaldehyde hydrazine, hydroxybenzophenone, 2-hydroxy-4-methoxybenzophenone, 4-methoxybenzophenone, 3,4-dihydroxybenzophenone, p-

anisic acid, p-anisaldehyde, 3,4-dihydroxybenzaldehyde, 3,4-dihydroxybenzoic acid, 2,5-dimethoxy-4-hydroxybenzaldehyde, 3,5-dimethoxy-4-hydroxybenzoic acid, 4-hydroxybenzaldehyde, salicylaldehyde, vanillin, vanillic acid.

APPENDIX 5

Possible Oxidation Reactions of the Enzyme Component System

1) Hydroxylation reactions

- a) Synthesis of alcohols
- b) Hydroxylation of steroids
- c) Hydroxylation of terpenes
- d) Hydroxylation of benzenes
- e) Hydroxylation of alkanes
- f) Hydroxylation of aromatic compounds
- g) Hydroxylation of double bonds
- h) Hydroxylation of nonactivated methyl groups
- i) Dihydroxylation of aromatic compounds

2) Oxidation of unsaturated aliphatics

- a) Preparation of epoxides
- b) Preparation of compounds by epoxidation
- c) Preparation of arene oxides
- d) Preparation of phenols
- e) Preparation of cis-dihydrodiols

3) Baeyer-Villiger oxidations

- a) Baeyer-Villiger conversion of steroids

4) Oxidation of heterocycles

- a) Transformation of organic sulfides
- b) Oxidation of sulfur compounds
- c) Oxidation of nitrogen compounds (formation of N-oxides etc.)
- d) Oxidation of other heteroatoms

5) Carbon-carbon dehydrogenation

- a) Dehydrogenation of steroids

6) Other oxidation reactions

- a) Oxidation of alcohols and aldehydes
- b) Oxidation of aromatic methyl groups to aldehydes
- c) Oxidative coupling of phenols
- d) Oxidative degradation of alkyl chains (β -oxidation etc.)
- e) Formation of peroxides or percompounds
- f) Initiation of free-radical induced chain reactions.